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(54) Title: DIAGNOSIS OF METASTATIC CANCER BY THE MTS-1 GENE		
(57) Abstract <p>The present invention is directed towards the diagnosis of malignant cancer by detection of the <u>mts-1</u> mRNA or the <u>mts-1</u> protein, encoded by the <u>mts-1</u> gene. The present invention contemplates the use of recombinant <u>mts-1</u> DNA and antibodies directed against the <u>mts-1</u> protein to diagnose the metastatic potential of several types of tumor cells, including, for example, thyroid, epithelial, lung, liver and kidney tumor cells. The present invention is also directed to mammalian cell lines and tumors with high and low metastatic potential which have been developed to serve as useful model systems for <u>in vitro</u> and <u>in vivo</u> anti-metastasis drug screening.</p>		

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1 DIAGNOSIS OF METASTATIC CANCER BY THE MTS-1 GENE

FIELD OF THE INVENTION

 The present invention is directed towards the
5 diagnosis of malignant cancer by detection of the mts-1
 mRNA or the mts-1 protein encoded by the mts-1 gene.
 The present invention contemplates the use of
 recombinant mts-1 DNA and antibodies directed against
10 the mts-1 protein to diagnose the metastatic potential
 of several types of tumor cells, including, for example,
 thyroid, epithelial, lung, liver, kidney, breast,
 lymphoid, hematopoietic, pancreatic, endometrial,
 ovarian, cervical, skin, colon and similar tumor cells.
 The present invention is also directed to mammalian cell
15 lines and tumors with high and low metastatic potential
 which have been developed to serve as useful model
 systems for in vitro and in vivo anti-metastasis drug
 screening.

20 BACKGROUND OF THE INVENTION

 Malignant cancer tumors shed cells which
 migrate to new tissues and create secondary tumors; a
 benign tumor does not generate secondary tumors. The
 process of generating secondary tumors is called
25 metastasis and is a complex process in which tumor cells
 colonize sites distant from the primary tumor. Tumor
 metastasis remains the major cause of morbidity and
 death for patients with cancer. One of the greatest
 challenges in cancer research is to understand the basis
30 of metastasis, i.e., what controls the spread of tumor
 cells through the blood and lymphatic systems and what

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1 allows tumor cells to populate and flourish in new
locations.

5 The metastatic process appears to be
sequential and selective, and is controlled by a series
of steps since metastatic tumor cells: (a) are mobile
and can disseminate from the original tumor; (b) are
capable of invading the cellular matrix and penetrating
10 through blood vessels; (c) possess immunological
markers, which allow them to survive passage through the
blood stream, where they must avoid the immunologically
active cytotoxic "T" lymphocytes; and (d) have the
ability to find a favorable location to transplant
themselves and successfully survive and grow.

15 Understanding the underlying molecular
mechanisms in metastasis is the key to understanding
cancer biology and its therapy. In clinical lesions,
malignant tumors contain a heterogeneous population of
cells, exhibiting a variety of biological
20 characteristics, e.g., differential growth rates, cell
surface structures, invasive capacities and sensitivity
to various cytotoxic drugs. Researchers can take
advantage of tumor heterogeneity factors, by identifying
specific cell produced markers, which are unique for
metastasis, to develop therapeutic regimens which do
25 not rely only on surgical resection.

At this time it is not known whether the
metastatic phenotype is under the regulation of a single
or multiple gene(s), and whether these genes are
independent or interrelated. However, a number of genes
30 have become correlated with the formation and metastasis
of tumors. For example, several normal cellular genes
become oncogenes by incorporation into a retroviral

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1 100 fold higher levels in metastatic cells compared to
non-metastatic cells and normal cells. Only a few types
of normal cells, including lymphocytes and trophoblasts,
express mts-1. Hence, the present invention
5 demonstrates a surprising new property of mts-1: the
misexpression of mts-1 within a cell or tissue is
diagnostic of malignant cancer.

SUMMARY OF THE INVENTION

10 The present invention is directed towards the
diagnosis of metastatic cancer using an mts-1 nucleic
acid or antibodies directed against the mts-1 protein.
The present invention is also directed to isolated and
purified mts-1 nucleic acids available for diagnostic
15 tests and antibodies directed against the mammalian mts-1
proteins.

One aspect of the present invention is
directed to a method for diagnosing metastatic cancer by
contacting serum from an individual to be tested for
20 such cancer with an antibody reactive with a mammalian
mts-1 protein or an antigenic fragment thereof, for a
time and under conditions sufficient to form an antigen-
antibody complex, and detecting the antigen-antibody
complex.

25 Another aspect of the present invention
provides an isolated, recombinant nucleic acid encoding
a human mts-1 gene or a fragment thereof, and replicable
DNA sequences encoding an mts-1 polypeptide which
express high levels of the mts-1 polypeptide. Isolated
30 antisense mts-1 nucleic acids and expression vectors
therefor are also contemplated by the present invention.
Human mts-1 nucleic acids are preferred.

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1 A further aspect of this invention is directed
to isolated transformed host cells, such as prokaryotic
microorganisms, yeast, insect cells and eukaryotic
cells, containing mts-1 nucleic acids and replicable
5 vectors containing DNA sequences encoding the mts-1
polypeptide.

 A still further aspect of this invention
provides isolated homogeneous mammalian mts-1
polypeptides and pharmaceutical compositions including
10 such a mts-1 polypeptide or protein. Human mts-1
polypeptides are preferred.

 Another aspect of this invention provides
antibodies directed against an mts-1 polypeptide or any
peptide, fragment or derivative of the mts-1 protein.

15 A further aspect of this invention is directed
towards treatment of cancer by administering reagents,
such as for example, anti-mts-1 antibodies capable of
binding the mts-1 protein and antisense mts-1 nucleic
acids capable of binding mts-1 sense mRNA.

20 Yet another aspect of the present invention
provides an animal model system of the metastatic
process, including several eukaryotic cell lines and
tumors expressing different levels of mts-1, which are
derived from mouse and rat carcinomas. These cell lines
25 and tumors may be re-introduced into mice or rats to
produce primary tumors which metastasize to the lung,
liver and kidneys with a characteristic frequency.
Therefore, the present invention also provides a well
controlled animal model system for testing
30 pharmaceutical compositions suspected to have
therapeutic utility for the treatment of metastatic
cancer.

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1 BRIEF DESCRIPTION OF THE DRAWINGS

 Fig. 1 depicts the nucleotide sequence of the coding region of the human mts-1 gene.

5 Fig. 2 depicts the amino acid sequence of the human mts-1 protein.

 Fig. 3 depicts the circular, expression plasmid pEMSVscribe2 containing the complete coding region of mts-1 under the control of the murine sarcoma virus promoter (MSV LTR).

10 Fig. 4 illustrates an autoradiograph showing detection of the mts-1 transcript by a mts-1 nucleic acid probe in a Northern blot of mRNA from a cell line with low metastatic potential (CSML-0) and a cell line with very high metastatic potential (CSML-100).

15 Fig. 5 illustrates an autoradiograph showing detection of the mts-1 transcript by a mts-1 nucleic acid probe in a Northern blot of mRNA from different metastatic (depicted with an "M" above the lane) and non-metastatic mouse tumors and cell lines. In the top
20 autoradiograph: Lane 1-HMC-Lr; Lane 2-HMC-0; Lane 3-RL-67; Lane 4-B-16, Lane 5-LLC; Lane 6-Acatol; Lane 7-C12; Lane 8-PCC4c-B; Lane 9-PCC4c-P, Lane 10-PCC4c-107; Lane 11-PCC4107; Lane 12-T9; Lane 13-LMEC; Lane 14-T36; Lane 15-T36cL. The bottom autoradiograph depicts the same
25 Northern blot hybridized with an actin probe, providing a comparison of the amounts of mRNA in each lane.

 Fig. 6 illustrates an autoradiograph showing detection of the mts-1 transcript by a mts-1 nucleic acid probe in a Northern blot of mRNA from various
30 tumors and tumor cell lines. Lanes 1 and 2-size markers; Lane 3-mouse lung carcinoma Line 1 grown without DMSO; Lane 4-mouse lung carcinoma Line 1 grown

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1 with 3% DMSO; Lane 5-IR6 tumor; Lane 6-TRCL, cell line;
Lane 7-IR6 cell line (IR6CL,); Lane 8-FRTL5 cell line.

Fig. 7 depicts a histopathological
characterization of some of the rat tumors of the
5 present invention, demonstrating the morphological and
histological identity of these tumors with corresponding
human tumors.

Fig. 8 illustrates an autoradiograph showing
detection of the mts-1 transcript by a mts-1 nucleic
10 acid probe in a Northern blot of mRNA from various Line
1 murine lung carcinoma cell lines containing a
transfected copy of the rat mts-1 gene (N1-N10), or just
an antibiotic resistance marker (Neo 1-3), all grown in
the presence of 3% DMSO; compared to Line 1 cells grown
15 without DMSO (Line 1). DMSO inhibits the development of
the metastatic phenotype as well as mts-1 expression in
non-transfected Line 1 cells, hence transfection of mts-1
can overcome this block.

Fig. 9a depicts the lungs from 3 mice injected
20 subcutaneously with 1×10^6 CSML-0 cells. Lungs were
removed 4-6 weeks after injection and then injected with
India ink. Dark areas indicate normal tissues; white
areas are tumors.

Fig. 9b depicts the lungs from 3 mice injected
25 intravenously with 1×10^4 CSML-0 cells. Lungs were
removed 15 days after injection and then injected with
India ink. Dark areas indicate normal tissues; white
areas are tumors.

Fig. 9c depicts the lungs from 3 mice injected
30 intravenously with 1×10^4 CSML-100 cells. Lungs were
removed 15 days after injection and then injected with

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1 India ink. Dark areas indicate normal tissues; white areas are tumors.

5 Fig. 9d depicts the lungs from 3 mice injected subcutaneously with 1×10^6 CSML-100 cells. Lungs were removed 4-6 weeks after injection and then injected with India ink. Dark areas indicate normal tissues; white areas are tumors.

10 Fig. 9e depicts the lungs from 3 mice injected with 0.1 ml serum-free media. Lungs were removed 6-8 weeks after injection and then injected with India ink. Dark areas indicate normal tissues; white areas are tumors.

15 Fig. 10a depicts a diagram of the more important regions of the pTrcHis B expression vector utilized to produce a histidine-mts-1 fusion protein. The murine mts-1 cDNA was subcloned into pTrcHis B at the BamHI-KpnI site to generate pTBM1.

20 Fig. 10b depicts a Coomassie Brilliant Blue-stained gel illustrating the profile of proteins eluted from a Ni²⁺-NTA column used to purify mts-1 protein expressed by cells containing pTBM1. Elution was with a series of buffers having pH values varying from 5.9 to 4.5. A single major protein, the mts-1 protein, is eluted.

25 Fig. 11 depicts a growth curve of CSML-0 and CSML-100 cells over a five day period. Cell growth was measured daily by observing the number of cells per dish (ordinate). As illustrated, CSML-100 cells, which express high levels of mts-1, grow at a slower rate than CSML-0 cells which express little mts-1.

30 Fig. 12a depicts a photomicrograph of a section from an 8 day mouse embryo hybridized with a ³²P-

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1 labelled mts-1 antisense probe. Signal is detected in
the trophoblast cells.

5 Fig. 12b depicts a photomicrograph of a
section from an 8 day mouse embryo hybridized with a ³²P-
labelled mts-1 sense probe. No signal is detected.

10 Fig. 13 depicts a western blot of CSML-0 (Lane
1) and CSML-100 (Lanes 2 and 3) cell lysates. Lanes 1
and 2 were probed with the chicken anti-mts-1 antibody
(α -mts-1) using a secondary antibody (rabbit anti-
chicken IgG-HRP) for detection. Lane 3 was similarly
15 probed except that free mts-1 protein was added during
the incubation with the α -mts-1 antibody. An
approximate 10-12 kd mts-1 protein is detected only in
CSML-100 cells and only when no free mts-1 protein is
20 present to compete for binding to the α -mts-1 antibody.
Therefore, the α -mts-1 antibody is highly specific for
mts-1 protein.

25 Fig. 14a depicts a frozen mouse spleen section
probed with the α -mts-1 antibody. Rabbit anti-chicken
IgG-HRP was used for detection of the mts-1 antigen-
antigen complex (dark spots).

30 Fig. 14b depicts a frozen mouse spleen section
probed with the α -mts-1 antibody in the presence of free
mts-1 protein. Rabbit anti-chicken IgG-HRP was used for
detection of the mts-1 antigen-antigen complex (dark
spots). As illustrated, little or no mts-1 protein is
detected when free mts-1 protein is present to compete
for binding to α -mts-1 (compare to Fig. 14a).
Therefore, the α -mts-1 antibody is highly specific for
35 mts-1 protein.

Fig. 15a illustrates that mts-1 protein can be
detected only in serum from mice injected with CSML-100

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1 cells. This figure depicts a western blot of serum
taken from non-injected mice (Lane 3), mice injected
with 1×10^5 CSML-0 cells (Lane 1) and mice injected
with 1×10^5 CSML-100 cells (Lane 2). After reaction
5 with the α -mts-1 antibody a 10-12 kd mts-1 protein is
detected only in the serum from mice injected with CSML-
100 cells. The higher molecular weight bands merely
cross-react with the anti-mts-1 antibody used and were
not mts-1 proteins.

10 Fig. 15b similarly illustrates that mts-1
protein can be detected only in serum from mice injected
with CSML-100 cells. This figure depicts a western blot
of serum taken from non-injected mice (Lane 3), mice
injected with 1×10^5 CSML-0 cells (Lane 1) and mice
15 injected with 1×10^5 CSML-100 cells (Lane 2). After
reaction with the α -mts-1 antibody a 10-12 kd mts-1
protein is detected only in the serum from mice injected
with CSML-100 cells. As described, the higher molecular
weight bands merely cross-react with the anti-mts-1
20 antibody used and were not mts-1.

Fig. 15c depicts a western blot of lysed whole
blood from mice probed with the α -mts-1 antibody. Lanes
1-4 were loaded with 5, 10, 20 and 25 μ l lysed whole
blood, respectively. Lane 5 was loaded with CSML-100
25 cell lysate as a positive control. This blot
illustrates that mts-1 protein in serum is not simply
due to lysis of lymphocyte or blood cells.

Fig. 15d depicts a western blot of increasing
amounts of serum from mice injected with salmonella
30 lipopolysaccharide (LPS) to induce a chronic immune
response. The blot was probed with the α -mts-1 antibody
to reveal any detectable mts-1 protein. Lanes 1-3 were

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1 loaded with 75, 100 or 150 μ g serum, respectively. This
blot illustrates that mts-1 protein in serum is not
derived from activated macrophages generated by a
chronic immune response.

5 Fig. 16 depicts a western blot of sera from
patients with non-metastatic and metastatic cancers
probed with the α -mts-1 antibody to reveal any
detectable mts-1 protein. A 27 kd mts-1 protein is
10 detected only in patients known to have metastatic
cancer. Sera were taken from patients with non-
metastatic breast cancer (Lane 1), with non-metastatic
lymphomas (Lanes 2 and 4), with metastatic lymphomas
(Lanes 5 and 7) and with metastatic breast cancer (Lane
6). Lane 3 contains normal serum as a negative control.
15 The higher molecular weight proteins merely cross-react
with the α -mts-1 antibody and do not represent mts-1
protein products.

DETAILED DESCRIPTION OF THE INVENTION

20 The present invention provides a new method
for diagnosing metastatic cancer and for distinguishing
metastatic tumors from benign tumors. In particular,
the present invention demonstrates a heretofore unknown
property of a mammalian gene, called mts-1, whose
25 expression is about 10 to about 100 fold higher in
metastatic tumor cells, for example, of the lung, liver,
kidney, mammary gland, epithelial, thyroid, leukemic,
pancreatic, endometrial, ovarian, cervical, skin, colon
or lymphoid tissue than in benign tumor cells or the
30 corresponding normal cells. According to the present
invention metastatic cancer of these and other tissues
can be detected in patient's serum by a simple

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1 immunoassay. Moreover, metastatic cancer can also be
diagnosed in tissue biopsies by the present immunoassays
or by in situ hybridization assays.

5 Metastasis is the formation of secondary
tumors by cells derived from a primary tumor. The
metastatic process involves mobilization and migration
of primary tumor cells from the site of the primary
tumor into new tissues where the primary tumor cells
induce the formation of secondary (metastatic) tumors.
10 In accordance with the present inventive discovery, the
increased expression of the mts-1 gene in a cell or
tissue is strongly indicative of metastatic potential.
The present invention utilizes this unexpected and
surprising correlation of high mammalian mts-1 gene
15 expression with high metastatic potential to detect or
diagnose malignant cancer. Both the mammalian mts-1
nucleic acid and antibodies directed against mammalian
mts-1 proteins are contemplated for use in the diagnosis
of malignant cancer. The human mts-1 gene, depicted by
20 one of the nucleotide sequences below, has been isolated
for the first time in the present invention.

SEQ ID NO:1

25 ATG-GCG-TGC-CCT-CTG-GAG-AAG-GCC-CTG-GAT-GTG-ATG-GTG-TCC-
ACC-TTC-CAC-AAG-TAC-TCG-GGC-AAA-GAG-GGT-GAC-AAG-TTC-AAG-
CTC-AAC-AAG-TCA-GAG-CTA-AAG-GAG-CTG-CTG-ACC-CGG-GAG-CTG-
CCC-AGC-TTC-TTG-GGG-AAA-AGG-ACA-GAT-GAA-GCT-GCT-TTC-CAG-
AAG-CTG-ATG-AGC-AAC-TTG-GAC-AGC-AAC-AGG-GAC-AAC-GAG-GTG-
GAC-TTC-CAA-GAG-TAC-TGT-GTC-TTC-CTG-TCC-TGC-ATC-GCC-ATG-
30 ATG-TGT-AAC-GAA-TTC-TTT-GAA-GGC-TTC-CCA-GAT-AAG-CAG-CCC-
AGG-AAG-AAA; or

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1 SEQ ID NO:3

GGC-AGT-TGA-GGC-AGG-AGA-CAT-CAA-GAG-AGT-ATT-TGT-GCC-
CTC-CTC-GGG-TTT-TAC-CTT-CCA-GCC-GAG-ATT-CTT-CCC-CTC-
TCT-ACA-ACC-CTC-TCT-CCT-CAG-CGC-TTC-TTC-TTT-CTT-GGT-
5 TTG-ATC-CTG-ACT-GCT-GTC-ATG-GCG-TGC-CCT-CTG-GAG-AAG-
GCC-CTG-GAT-GTG-ATG-GTG-TCC-ACC-TTC-CAC-AAG-TAC-TCG-
GGC-AAA-GAG-GGT-GAC-AAG-TTC-AAG-CTC-AAC-AAG-TCA-GAA-
CTA-AAG-GAG-CTG-CTG-ACC-CGG-GAG-CTG-CCC-AGC-TTC-TTG-
10 GGG-AAA-AGG-ACA-GAT-GAA-GCT-GCT-TTC-CAG-AAG-CTG-ATG-
AGC-AAC-TTG-GAC-AGC-AAC-AGG-GAC-AAC-GAG-GTG-GAC-TTC-
CAA-GAG-TAC-TGT-GTC-TTC-CTG-TCC-TGC-ATC-GCC-ATG-ATG-
TGT-AAC-GAA-TTC-TTT-GAA-GGC-TTC-CCA-GAT-AAG-CAG-CCC-
AGG-AAG-AAA-TGA-AAA-CTC-CTC-TGA-TGT-GGT-TGG-GGG-GTC-
15 TGC-CAG-CTG-GGG-CCC-TCC-CTG-TCG-CCA-GTG-GGC-ACT-TTT-
TTT-TTT-CCA-CCC-TGG-CTC-CTT-CAG-ACA-CGT-GCT-TGA-TGC-
TGA-GCA-AGT-TCA-ATA-AAG-ATT-CTT-GGA-AGT-TTA,

wherein SEQ ID NO:3 is different from SEQ ID NO:1 at the underlined positions.

20 The amino acid sequence of the human mts-1 protein is depicted below (SEQ ID NO:2):

Met-Ala-Cys-Pro-Leu-Glu-Lys-Ala-Leu-Asp-Val-Met-Val-Ser-
Thr-Phe-His-Lys-Tyr-Ser-Gly-Lys-Glu-Gly-Asp-Lys-Phe-Lys-
Leu-Asn-Lys-Ser-Glu-Leu-Lys-Glu-Leu-Leu-Thr-Arg-Glu-Leu-
25 Pro-Ser-Phe-Leu-Gly-Lys-Arg-Thr-Asp-Glu-Ala-Ala-Phe-Gln-
Lys-Leu-Met-Ser-Asn-Leu-Asp-Ser-Asn-Arg-Asp-Asn-Glu-Val-
Asp-Phe-Gln-Glu-Tyr-Cys-Val-Phe-Leu-Ser-Cys-Ile-Ala-Met-
Met-Cys-Asn-Glu-Phe-Phe-Glu-Gly-Phe-Pro-Asp-Lys-Gln-Pro-
Arg-Lys-Lys.

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Other mammalian mts-1 genes are also contemplated.

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1 The present invention also relates to a useful
animal model system of metastasis for screening
potential antimetastatic drugs and for developing
therapeutic regimens for cancer treatment. This model
5 system includes non-metastasizing and metastasizing
tumors that are maintained by sequential transplantation
from one mouse or rat to another, as well as cultured
cell lines, derived from these tumors, which retain the
metastatic or non-metastatic potential of their parental
10 tumors. Hence, these tumors or cell lines may be
transplanted or injected into mice or rats to generate
benign or metastatic tumors. Concurrently, drugs or
other therapies with anti-tumorigenic or anti-
metastatic potential, may be introduced into the animal
15 to test whether the formation of the metastatic and
benign tumors is suppressed. This model system has high
utility because of the predictable metastatic potential
of the tumors and cell lines therein and also because
cell lines of differing metastatic potential were
20 derived from the same parental tumor and hence have a
common genetic and phenotypic make-up, except for their
metastatic potential. Hence the animal model system of
the current invention is highly controlled and has
predictable metastatic potential.

25 The human mts-1 gene of the present invention
was obtained by use of mouse and rat mts-1 clones
previously obtained by the present inventors. The mouse
and rat mts-1 genes were obtained from cDNA libraries
made from metastatic mouse and rat tumor RNAs. The
30 mouse mts-1 gene has been obtained from a highly
metastatic cell line derived from a spontaneous mouse
mammary carcinoma (CSML-100), while the mts-1 rat gene

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1 utilized in the present invention was from a highly
metastatic thyroid carcinoma, IR-6. Both the mouse and
rat mts-1 genes were obtained by differential
hybridization of the respective cDNA libraries with a
5 probe made from a pool of mRNAs from highly metastatic
tissues, and a probe made from a pool of mRNAs from low
metastatic tissues.

The human mts-1 gene was obtained from a cDNA
library made by the present inventors from mRNA purified
10 from cultured HeLa cells and from cultured melanoma Wm64
cells. Clones hybridizing strongly to a mouse mts-1
cDNA probe can be identified as being the human mts-1
homologue by DNA sequencing. Alternatively, a cDNA can
be obtained by reverse transcription and polymerase
15 chain reaction using mRNA purified from metastatic
cells, e.g. as provided in Miller 1988 Ann. Rev.,
Microbiol. 42: 177.

There is a difference of seven amino acids
between the mouse and human mts-1 proteins,
20 demonstrating that while the mouse and human proteins
are functionally related they are not identical
structurally.

In another embodiment, the mouse, rat, and, in
particular, the human mts-1 genes of the present
25 invention have been subcloned into convenient replicable
vectors for production of large amounts of mts-1 DNA and
large amounts of sense or antisense mts-1 RNA.
Convenient replicable vectors comprise the gene or a DNA
fragment thereof of the present invention, an origin of
30 replication which is operable in the contemplated host,
and, preferably, a selectable marker, for example, an
antibiotic resistance marker. Many of these vectors are

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1 based on pBR322. Convenient replicable vectors which
allow synthesis of RNA from the DNA of interest include
Bluescript™ (commercially available from Stratagene),
5 pTrcHisB (Invitrogen) and others that are well known in
the art.

The present invention also contemplates
replicable expression vectors allowing a higher level of
expression of the mammalian mts-1 protein. Replicable
expression vectors as described herein are generally DNA
10 molecules engineered for controlled expression of a
desired gene, especially high level expression where it
is desirable to produce large quantities of a particular
gene product, or polypeptide. The vectors encode
15 promoters and other sequences to control expression of
that gene, the gene being expressed, and an origin of
replication which is operable in the contemplated host.
Preferably the vector also encodes a selectable marker,
for example, antibiotic resistance. Replicable
expression vectors can be plasmids, bacteriophages,
20 cosmids and viruses. Any expression vector comprising
RNA is also contemplated.

Preferred vectors of the present invention are
derived from eukaryotic sources. Expression vectors
that function in tissue culture cells are especially
25 useful, but yeast vectors are also contemplated. These
vectors include yeast plasmids and minichromosomes,
retrovirus vectors, BPV (bovine papilloma virus)
vectors, baculovirus vectors, SV40 based vectors and
other viral vectors. SV40-based vectors and retrovirus
30 vectors (e.g., murine leukemia viral vectors) are
preferred. Tissue culture cells that are used with
eukaryotic replicable expression vectors include Sf21

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1 cells, CV-1 cells, COS-1 cells, NIH3T3 cells, mouse L
cells, HeLa cells and such other cultured cell lines
known to one skilled in the art.

5 A baculovirus expression system can be used to
produce large amounts of mts-1 polypeptides in cultured
insect cells. The post-translational processing of
polypeptides produced in such insect cells is similar to
that of mammalian cells. Production of polypeptides in
10 insects is therefore advantageous, particularly when one
seeks to mimic the exact function or antigenic
properties of the natural polypeptide. Moreover, mts-1
polypeptides expressed in the baculovirus system are
produced without the need for a fused heterologous
polypeptide because the mts-1 start codon is used as the
15 translational start site.

Methods for producing polypeptides in the
baculovirus expression system are known to the skilled
artisan. See for example Miller 1988 Ann. Rev.
Microbiol. 42: 177. In general, a modified Autographa
20 californica nuclear polyhedrosis virus propagated in
Sf21 cells is used for polypeptide expression. This
modified virus is produced by cotransfection of a small
transfer vector, encoding an mts-1 polypeptide, with a
viral expression vector which has been linearized within
25 an essential gene. Once inside the cell, the linearized
expression vector can undergo recombination with the
transfer vector or simply recircularize. However, only
recombination gives rise to viable viruses because the
function of the essential gene is lost by
30 recircularization. Recombinant expression viruses are
detected by formation of plaques. The present
invention also contemplates prokaryotic vectors that may

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1 be suitable for expression of the mammalian mts-1 gene,
including bacterial and bacteriophage vectors that can
transform such hosts as E. coli, B. subtilis,
5 Streptomyces sps. and other microorganisms. Many of
these vectors are based on pBR322 including Bluescript™
(commercially available from Stratagene) and are well
known in the art. Bacteriophage vectors that are used
in the invention include lambda and M13.

Sequence elements capable of effecting
10 expression of the human mts-1 gene include promoters,
enhancer elements, transcription termination signals and
polyadenylation sites. Promoters are DNA sequence
elements for controlling gene expression, in particular,
they specify transcription initiation sites.
15 Prokaryotic promoters that are useful include the lac
promoter, the trp promoter, and P_L and P_R promoters of
lambda and the T7 polymerase promoter. Eukaryotic
promoters are especially useful in the invention and
include promoters of viral origin, such as the SV40 late
20 promoter and the Moloney Leukemia Virus LTR, Murine
Sarcoma Virus (MSV) LTR, yeast promoters and any
promoters or variations of promoters designed to control
gene expression, including genetically-engineered
promoters. Control of gene expression includes the
25 ability to regulate a gene both positively and
negatively (i.e., turning gene expression on or off) to
obtain the desired level of expression.

The replicable expression vectors of the
present invention can be made by ligating part or all of
30 the mts-1 coding region in the sense or antisense
orientation to the promoter and other sequence elements
being used to control gene expression. This

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1 juxtapositioning of promoter and other sequence elements
with the mts-1 gene allows the production of large
amounts of sense or antisense mts-1 mRNA. Large amounts
of the mts-1 protein can also be produced which are
5 useful not only for anti-mts-1 antibody production but
also for analysis of the function of mts-1 in metastatic
cancer as well as for designing therapies for metastatic
cancer.

As one example of an appropriate expression
10 vector for the human mts-1 gene, the present invention
provides the pEMSVscribe2 vector which expresses the
human mts-1 gene of this invention.

In another example, large quantities of the
mts-1 specific protein were expressed in an E. coli host
15 using the inducible bacterial vector pTrcHisB (Fig.
10a). Murine mts-1 cDNA was subcloned in frame into a
BamHI-KpnI site with the multiple cloning site of
pTrcHisB to generate plasmid pTBM1. The fusion protein
expressed by pTBM1 has 6 tandem histidine residues which
20 allow easy purification of the fusion protein because of
the high affinity of such tandem histidines for a Ni^{++}
charged resin. The fusion protein also has an
enterokinase specific cleavage site permitting removal
of the histidines from the mts-1 protein product.
25 Expression of the mts-1 fusion protein encoded by pTBM1
can be induced by IPTG. Similar human mts-1 cDNA
constructs have also been generated.

Therefore, one skilled in the art has
available many choices of replicable expression vectors,
30 compatible hosts and well-known methods for making and
using the vectors. Recombinant DNA methods are found in

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1 any of the myriad of standard laboratory manuals on
genetic engineering.

The present invention is also directed to the
detection of metastatic cancer in tissue specimens by
5 use of the mts-1 DNA as a nucleic acid probe for
detection of mts-1 mRNA, or by use of antibodies
directed against the mts-1 protein.

The nucleic acid probe of the present
invention may be any portion or region of a mammalian
10 mts-1 RNA or DNA sufficient to give a detectable signal
when hybridized to mts-1 mRNA derived from a tissue
sample. The nucleic acid probe produces a detectable
signal because it is labeled in some way, for example
because the probe was made by incorporation of
15 nucleotides linked to a "reporter molecule".

A "reporter molecule", as used in the present
specification and claims, is a molecule which, by its
chemical nature, provides an analytically identifiable
signal allowing detection of the hybridized probe.
20 Detection may be either quantitative or quantitative.
The most commonly used reporter molecules in this type
of assay are either enzymes, fluorophores or
radionuclides covalently linked to nucleotides which are
incorporated into a mts-1 DNA or RNA. Commonly used
25 enzymes include horseradish peroxidase, alkaline
phosphatase, glucose oxidase and β -galactosidase, among
others. The substrates to be used with the specific
enzymes are generally chosen for the production, upon
hydrolysis by the corresponding enzyme, of a detectable
30 color change. For example, p-nitrophenyl phosphate is
suitable for use with alkaline phosphatase conjugates;

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1 for horseradish peroxidase, 1,2-phenylenediamine, 5-aminosalicylic acid or toluidine are commonly used.

5 Incorporation into a mts-1 DNA probe may be by nick translation, random oligo priming, by 3' or 5' end labeling, by labeled single-stranded DNA probes using single-stranded bacteriophage vectors (e.g. M13 and related phage), or by other means, (Sambrook et al., 1989, Molecular Cloning, A Laboratory Manual. Cold Spring Harbor Laboratory Press. Pages 10.1-10.70).

10 Incorporation of a reporter molecule into a mts-1 RNA probe may be by synthesis of mts-1 RNA using T3, T7, Sp6 or other RNA polymerases (Sambrook et al., supra: 10.27-10.37).

15 Detection or diagnosis of metastatic cancer by the nucleic acid probe of the present invention can be by a variety of hybridization techniques which are well known in the art. In one embodiment, patient tissue specimens are sectioned and placed onto a standard microscope slide, then fixed with an appropriate
20 fixative. The mts-1 RNA or DNA probe, labeled by one of the techniques described above, is added. The slide is then incubated at a suitable hybridization temperature (generally 37°C to 55°C) for 1-20 hours. Non-hybridized RNA or DNA probe is then removed by extensive, gentle
25 washing. If a non-radioactive reporter molecule is employed in the probe, the suitable substrate is applied and the slide incubated at an appropriate temperature for a time appropriate to allow a detectable color signal to appear as the slide is visualized under light
30 microscopy. Alternatively, if the mts-1 probe is labeled radioactively, slides may be dipped in photoemulsion after hybridization and washing, and the

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1 signal detected under light microscopy after several days, as exposed silver grains.

Metastatic cancer can also be detected from RNA derived from tissue specimens by the mts-1 nucleic acid probe. RNA from specimens can be fixed onto nitrocellulose or nylon filters, and well-known filter hybridization techniques may be employed for detection of mts-1 gene expression. Specimen mRNA can be purified, or specimen cells may be simply lysed and cellular mRNA fixed unto a filter. Specimen mRNA can be size fractionated through a gel before fixation onto a filter, or simply dot blotted unto a filter.

In another embodiment, the mts-1 nucleic acid detection system of the present invention also relates to a kit for the detection of mts-1 mRNA. In general, a kit for detection of mts-1 mRNA contains at least one mts-1 nucleic acid. Such an mts-1 nucleic acid can be a probe having an attached reporter molecule or the mts-1 nucleic acid can be unlabelled. The unlabelled mts-1 nucleic acid can be modified by the kit user to include a reporter molecule or can act as a substrate for producing a labelled mts-1 probe, for example by nick translation or RNA transcription.

In another embodiment, the kit is compartmentalized: a first container can contain mts-1 RNA at a known concentration to act as a standard or positive control, a second container can contain mts-1 DNA suitable for synthesis of a detectable nucleic acid probe, and a third and a fourth container can contain reagents and enzymes suitable for preparing said mts-1 detectable probe. If the detectable nucleic acid probe is made by incorporation of an enzyme reporter molecule,

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1 a fifth or sixth container can contain a substrate, or
substrates, for the enzyme provided.

5 In accordance with the present invention, the
mts-1 protein or portions thereof can be used to
generate antibodies useful for the detection of the mts-
10 1 protein in clinical specimens. Such antibodies may be
monoclonal or polyclonal. Additionally, it is within
the scope of this invention to include second antibodies
(monoclonal or polyclonal) directed to the anti-mts-1
antibodies. The present invention further contemplates
use of these antibodies in a detection assay
(immunoassay) for the mts-1 gene product.

15 The present invention further contemplates
antibodies directed against the mammalian, including
rat, mouse and human, mts-1 proteins or polypeptides.
These antibodies may be generated by using the entire
mts-1 protein as an antigen or by using short peptides,
encoding portions of the mts-1 protein, as antigens.
When peptides are contemplated they have at least about
20 4 amino acids and preferably at least about 10 amino
acids.

25 Preferably, specific peptides encoding unique
portions of the mammalian mts-1 gene are synthesized for
use as antigens for obtaining mts-1 antibodies. This is
done because mts-1 encodes a calcium binding domain
whose sequence, and hence antigenicity, is similar to
other calcium binding proteins. By utilizing peptides
encoding sequences lying outside the calcium binding
domain, cross-reactivity of the anti-mts-1 antibodies
30 towards other calcium binding proteins easily can be
avoided. Accordingly, peptide sequences are tested for
sequence homologies by searching protein sequence data

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1 banks before peptides are actually synthesized. Among
the various mts-1 peptides that can be used, four
peptides encoding a portion of the human mts-1 sequence
shown below, have already been used to generate
5 antibodies:

- 1) Unique peptide encoding amino acids 2-11 of
the mts-1 protein (SEQ ID NO:4):
Ala-Cys-Pro-Leu-Glu-Lys-Ala-Leu-Asp-Val;
- 10 2) Peptide encoding the calcium binding domain of
the mts-1 protein (amino acids 22-37, SEQ ID
NO:5):
Lys-Glu-Gly-Asp-Lys-Phe-Lys-Leu-Asn-Lys-Ser-
Glu-Leu-Lys-Glu-Leu;
- 15 3) Unique peptide encoding amino acids 42-54 of
the mts-1 protein (SEQ ID NO:6):
Leu-Pro-Ser-Phe-Leu-Gly-Lys-Arg-Thr-Asp-Glu-
Ala-Ala;
- 20 4) Unique peptide encoding amino acids 87-101 of
mts-1 protein (SEQ ID NO:7):
Asn-Glu-Phe-Phe-Glu-Gly-Phe-Pro-Asp-Lys-Gln-
Pro-Arg-Lys-Lys.

25 Polyclonal antibodies directed against the
mts-1 protein are prepared by injection of a suitable
laboratory animal with an effective amount of the
peptide or antigenic component, collecting serum from
the animal, and isolating specific sera by any of the
known immunoadsorbent techniques. Animals which can
30 readily be used for producing polyclonal anti-mts-1
antibodies include chickens, mice, rabbits, rats, goats,
horses and the like. Chickens are preferred because a

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1 better immune response can be obtained and because
antibodies can be collected from eggs rather than by
bleeding. Although the polyclonal antibodies produced
by this method are utilizable in virtually any type of
5 immunoassay, they are generally less favored because of
the potential heterogeneity of the product.

The use of monoclonal antibodies in the
diagnostic or detection assays of the present invention
is particularly preferred because large quantities of
10 antibodies, all of similar reactivity, may be produced.
The preparation of hybridoma cell lines for monoclonal
antibody production is done by fusing an immortal cell
line and the antibody producing lymphocytes. This can
be done by techniques which are well known to those who
15 are skilled in the art. (See, for example, Harlow, E.
and Lane, D., Antibodies: A Laboratory Manual, Cold
Spring Harbor Press, 1988; or Douillard, J. Y. and
Hoffman, T., "Basic Facts About Hybridomas", in
Compendium of Immunology Vol. II, L. Schwartz (Ed.),
20 1981.

Unlike the preparation of polyclonal sera, the
choice of animal for monoclonal antibody preparation is
dependent on the availability of appropriate immortal
cell lines capable of fusing with the monoclonal
25 antibody producing lymphocytes derived from the
immunized animal. Mouse and rat have been the animals
of choice for hybridoma technology and are preferably
used. Humans can also be utilized as sources for
antibody producing lymphocytes if appropriate
30 immortalized human (or nonhuman) cell lines are
available. For the purpose of making the monoclonal
antibodies of the present invention, the animal of

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1 choice may be injected with from about 0.01 mg to about
20 mg of the purified mts-1 antigen. Usually the
injecting material is emulsified in Freund's complete
5 adjuvant. Boosting injections are generally also
required. The separate immortalized cell lines obtained
by cell fusion may be tested for antibody production by
testing the cell culture media for the ability to find
the appropriate antigen.

10 Lymphocytes can be obtained by removing the
spleen or lymph nodes of immunized animals in a sterile
fashion. Alternately, lymphocytes can be stimulated or
immunized in vitro, as described, for example, in C.
Reading J. Immunol. Meth. 53:261-291 1982. To
15 immortalize the monoclonal antibody producing
lymphocytes, the lymphocytes must be fused to
immortalized cells. A number of cell lines suitable for
fusion have been developed, and the choice of any
particular line for hybridization protocols is directed
by any one of a number of criteria such as speed,
20 uniformity of growth characteristics, deficiency of its
metabolism for a component of the growth medium, and
potential for good fusion frequency. Intraspecies
hybrids, particularly between like strains, work better
than interspecies fusions.

25 Several cell lines are available, including
mutants selected for the loss of ability to create
myeloma immunoglobulin. Included among these are the
following mouse myeloma lines: MPC,, -X45-6TG, P3 NS1/1-
Ag4-1, P3-X63-Ag14 (all BALB/C derived), Y3'Ag1.2.3
30 (rat), and U266 (human).

Cell fusion can be induced either by virus,
such as Epstein-Barr or Sendai virus, or polyethylene

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1 glycol. Polyethylene glycol (PEG) is the most
efficacious agent for the fusion of mammalian somatic
cells. PEG itself may be toxic for cells, and various
concentrations should be tested for effects on viability
5 before attempting fusion. The molecular weight range of
PEG may be varied from 1,000 to 6,000. It give best
results when diluted to from about 20% to about 70% w/w
in saline or serum-free medium. Exposure to PEG at 37°C
for about 30 seconds is preferred in the present case,
10 utilizing murine cells. Extremes of temperature (i.e.
about 45°C) are avoided, and preincubation of each
component of the fusion system at 37°C prior to fusion
gives optimum results. The ratio between lymphocytes
and immortalized cells optimized to avoid cell fusion
15 amongst lymphocytes ranges of from about 1:1 to about
1:10.

The successfully fused cells can be separated
from the immortalized cell line by any technique known
by the art. The most common and preferred method is to
20 choose an immortalized cell line which is Hypoxanthine
Guanine Phosphoribosyl Transferase (HGPRT) deficient.
Since these cells will not grow in an aminopterin-
containing medium, only hybrids of lymphocytes and
immortalized cells will grow. The aminopterin-
25 containing medium is generally composed of hypoxanthine
 1×10^{-4} M, aminopterin 1×10^{-3} M, and thymidine
 3×10^{-3} M, commonly known as the HAT medium. Fused
cells are generally grown for two weeks and then fed
with either regular culture medium or hypoxanthine,
30 thymidine- containing medium.

The fused cell colonies are then tested for
the presence of antibodies that recognize the mts-1

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1 protein. Detection of hybridoma antibodies can be
performed using an assay where the antigen is bound to a
solid support and allowed to react to hybridoma
supernatants containing putative antibodies. The
5 presence of antibodies may be detected by "sandwich"
techniques using a variety of indicators. Most of the
common methods are sufficiently sensitive for use in the
range of antibody concentrations secreted during hybrid
growth.

10 Cloning of hybrid cells can be carried out
after 20-25 days of cell growth in selected medium.
Cloning can be performed by cell limiting dilution in
fluid phase or by directly selecting single cells
growing in semi-solid agarose. For limiting dilution,
15 cell suspensions are diluted serially to yield a
statistical probability of having only one cell per
well. For the agarose techniques, hybrids are seeded in
a semisolid upper layer, over a lower layer containing
feeder cells. The colonies from the upper layer may be
20 picked up and eventually transferred to wells.

Antibody-secreting hybrid cells can be grown
in various tissue culture flasks, yielding supernatants
with variable concentrations of antibodies. In order to
obtain higher concentrations, hybrid cells may be
25 transferred into animals to obtain inflammatory ascites.
Antibody- containing ascites can be harvested 8-12 days
after intraperitoneal injection. The ascites contain a
higher concentration of antibodies but include both
monoclonals and immunoglobulins from the inflammatory
30 ascites. Antibody purification may then be achieved by,
for example, affinity chromatography.

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1 One embodiment of the present invention is
directed to a method for diagnosing metastatic cancer by
contacting or applying an antibody reactive with an mts-
5 1 polypeptide to a tissue or blood sample taken from an
individual to be tested for metastatic cancer.
Formation of an antigen-antibody complex in this
immunoassay is diagnostic of metastatic cancer.

 In a preferred embodiment, the present
invention provides a method for diagnosing metastatic
10 cancer which involves contacting serum from an
individual to be tested for such cancer with an antibody
reactive with a mammalian mts-1 protein or an antigenic
fragment thereof, for a time and under conditions
sufficient to form an antigen-antibody complex, and
15 detecting the antigen-antibody complex.

 The presence of the mts-1 protein, or its
antigenic components, in a patient's serum, tissue or
biopsy sample can be detected utilizing antibodies
prepared as above, either monoclonal or polyclonal, in
20 virtually any type of immunoassay. A wide range of
immunoassay techniques are available as can be seen by
reference to Harlow, et al. (Antibodies: A Laboratory
Manual, Cold Spring Harbor Press, 1988) and U.S. Patent
Nos. 4,016,043 and 4,424,279. This, of course, includes
25 both single-site and two-site, or "sandwich" of the non-
competitive types, as well as in traditional competitive
binding assays. Sandwich assays are among the most
useful and commonly used assays. A number of variations
of the sandwich assay technique exist, and all are
30 intended to be encompassed by the present invention.
Briefly, in a typical forward assay, an unlabeled
antibody is immobilized in a solid substrate and the

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1 sample to be tested brought into contact with the bound
molecule. After a suitable period of incubation, for a
period of time sufficient to allow formation of an
antibody-antigen binary complex, a second antibody,
5 labeled with a reporter molecule capable of producing a
detectable signal is then added and incubated, allowing
time sufficient for the formation of a ternary complex of
antibody-labeled antibody. Any reacted material is
washing away, and the presence of the antigen is
10 determined by observation of a signal produced by the
reporter molecule. The results may either be
qualitative, by simple observation of the visible
signal, or may be quantitated by comparing with a
control sample containing known amounts of hapten.
15 Variations on the forward assay include a simultaneous
assay, in which both sample and labeled antibody are
added simultaneously to the bound antibody, or a reverse
assay in which the labeled antibody and sample to be
tested are first combined, incubated and then added to
20 the unlabeled surface bound antibody. These techniques
are well known to those skilled in the art, and then
possibly of minor variations will be readily apparent.
As used herein, "sandwich assay" is intended to
encompass all variations on the basic two-site
25 technique.

The mts-1 protein may also be detected by a
competitive binding assay in which a limiting amount of
antibody specific for the mts-1 protein is combined with
specified volumes of samples containing an unknown
30 amounts of the mts-1 protein and a solution containing a
detectably labeled known amount of the mts-1 protein.
Labeled and unlabeled molecules then compete for the

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1 available binding sites on the antibody. Phase
separation of the free and antibody-bound molecules
allows measurement of the amount of label present in
each phase, thus indicating the amount of antigen or
5 haptin in the sample being tested. A number of
variations in this general competitive binding assays
currently exist.

In any of the known immunoassays, for
practical purposes, one of the antibodies or the antigen
10 will be typically bound to a solid phase and a second
molecule, either the second antibody in a sandwich
assay, or, in a competitive assay, the known amount of
antigen, will bear a detectable label or reporter
molecule in order to allow visual detection of an
15 antibody-antigen reaction. When two antibodies are
employed, as in the sandwich assay, it is only necessary
that one of the antibodies be specific for the mts-1
protein or its antigenic components. The following
description will relate to a discussion of a typical
20 forward sandwich assay; however, the general techniques
are to be understood as being applicable to any of the
contemplated immunoassays.

In the typical forward sandwich assay, a first
antibody having specificity for the mts-1 protein or its
25 antigenic components is either covalently or passively
bound to a solid surface. The solid surface is
typically glass or a polymer, the most commonly used
polymers being cellulose, polyacrylamide, nylon,
polystyrene, polyvinyl chloride or polypropylene. The
30 solid supports may be in the form of tubes, beads, discs
or microplates, or any other surface suitable for
conducting an immunoassay. The binding processes are

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1 well-known in the art and generally consist of cross-
linking covalently binding or physically adsorbing the
molecule to the insoluble carrier. Following binding,
the polymer-antibody complex is washed in preparation
5 for the test sample. An aliquot of the sample to be
tested is then added to the solid phase complex and
incubated at a suitable temperature ranging from about
4°C to about 37°C (for example 25°C) for a period of
time sufficient to allow binding of any subunit present
10 in the antibody. The incubation period will vary but
will generally be in the range of about 2-40 minutes to
several hours. Following the incubation period, the
antibody subunit solid phase is washed and dried and
incubated with a second antibody specific for a portion
15 of a mts-1 hapten. The second antibody is linked to a
reporter molecule which is used to indicate the binding
of the second antibody to the hapten.

By "reporter molecule", as used in the present
specification and claims, is meant a molecule which, by
20 its chemical nature, provides an analytically
identifiable signal which allows the detection of
antigen-bound antibody. Detection may be either
qualitative or quantitative. The most commonly used
reporter molecules in this type of assay are either
25 enzymes, fluorophores or radionuclide containing
molecules. In the case of an enzyme immunoassay, an
enzyme is conjugated to the second antibody, generally
by means of glutaraldehyde or periodate. As will be
readily recognized, however, a wide variety of different
30 conjugation techniques exist, which are readily
available to the skilled artisan. Commonly used enzymes
include horseradish peroxidase, glucose oxidase, β -

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1 galactosidase and alkaline phosphates, among others.
The substrates to be used with the specific enzymes are
generally chosen for the production, upon hydrolysis by
the corresponding enzyme, of a detectable color change.
5 For example, p-nitrophenyl phosphate is suitable for use
with alkaline phosphatase conjugates; for peroxidase
conjugates, 1,2-phenylenediamine, 5-aminosalicylic acid,
or toluidine are commonly used. It is also
possible to employ fluorogenic substrates, which yield a
10 fluorescent product rather than the chromogenic
substrates noted above. In all cases, the enzyme-
labeled antibody is added to the first antibody hapten
complex, allowed to bind, and then the excess reagent is
washed away. A solution containing the appropriate
15 substrate is then added to the ternary complex of
antibody-antigen-antibody. The substrate will react
with the enzyme linked to the second antibody, giving a
qualitative visual signal, which may be further
quantitated, usually spectrophotometrically, to give an
20 indication of the amount of hapten which was present in
the sample.

Alternately, fluorescent compounds, such as
fluorescein and rhodamine, may be chemically coupled to
antibodies without altering their binding capacity.
25 When activated by illumination with light of a
particular wavelength, the fluorochrome-labeled antibody
absorbs the light energy, inducing a state of
excitability in the molecule, followed by emission of
the light at a characteristic color visually detectable
30 with a light microscope. The fluorescent labeled
antibody is allowed to bind to the first antibody-hapten
complex. After washing off the unbound reagent, the

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1 remaining ternary complex is then exposed to the light
of the appropriate wavelength, the fluorescence observed
indicates the presence of the hapten of interest.

Immunofluorescence techniques are very well established
5 in the art. However, other reporter molecules, such as
radioisotope, chemiluminescent or bioluminescent
molecules, may also be employed. It will be readily
apparent to the skilled technician how to vary the
procedure to suit the required purpose.

10 In another embodiment, the antibodies directed
against the mts-1 protein may be incorporated into a kit
for the detection of the mts-1 protein. Such a kit may
encompass any of the detection systems contemplated and
described herein, and may employ either polyclonal or
15 monoclonal antibodies directed against the mts-1
protein. Both mts-1 antibodies complexed to a solid
surface described above or soluble mts-1 antibodies are
contemplated for use in a detection kit. A kit of the
present invention has at least one container having an
20 antibody reactive with a mammalian mts-1 polypeptide.
However, the present kits can have other components.
For example, the kit can be compartmentalized: the
first container contains mts-1 protein as a solution, or
bound to a solid surface, to act as a standard or
25 positive control, the second container contains anti-
mts-1 primary antibodies either free in solution or
bound to a solid surface, a third container contains a
solution of secondary antibodies covalently bound to a
reporter molecule which are reactive against either the
30 primary antibodies or against a portion of the mts-1
protein not reactive with the primary antibody. A
fourth and fifth container contains a substrate, or

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1 reagent, appropriate for visualization of the reporter
molecule.

5 The subject invention therefore encompasses
polyclonal and monoclonal antibodies useful for the
detection of mts-1 protein as a means of diagnosing
metastatic cancer. Said antibodies may be prepared as
described above, then purified, and the detection
systems contemplated and described herein employed to
implement the subject invention.

10 The present invention also contemplates
treating metastatic cancers and tumors by inactivating,
destroying or nullifying the mts-1 gene or protein, or
cells expressing the mts-1 gene. The treatment of
cancer, as described in the specification and claims,
15 contemplates preferably lung, liver, kidney, thyroid,
mammary gland, leukemic, pancreatic, endometrial,
ovarian, cervical, skin, colon or lymphoid cancers. For
example, the antibodies, prepared as described above,
may be utilized to inactivate mts-1 protein expressing
20 cells: either unconjugated anti-mts-1 antibodies or
anti-mts-1 antibodies conjugated to a toxin may be
employed in the therapy of cancer.

Moreover, the present invention provides a
method of inhibiting metastasis in a cancerous cell by
25 providing to the cancerous cell a nucleic acid encoding
an antisense mts-1 nucleotide sequence. For example,
such an antisense nucleic acid can have at least 10
nucleotides of the antisense strand of SEQ ID NO:1 or
SEQ ID NO:3. Preferably, the antisense mts-1 nucleic
30 acids of the present invention have at least 15 or 17
nucleotides.

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1 In one embodiment, this method employs an
expression vector including a nucleic acid encoding an
antisense nucleotide sequence for mts-1 operably linked
to a segment of the vector which can effect expression
5 of an antisense mts-1 RNA. Any of the foregoing
expression vectors which can express high levels of mts-1
1 RNA can be used for this method including, e.g.,
pTrcHis.

According to the present invention, antisense
10 mts-1 nucleic acids can inhibit metastatic cancer by
binding to sense mts-1 mRNA. Such binding can either
prevent translation of mts-1 protein or destroy mts-1
sense mRNA, e.g., through the action of RNaseH.
Accordingly, less mts-1 protein is available to
15 potential metastatic tumor cells and metastasis of these
cells is prevented.

Another embodiment of the present invention
contemplates pharmaceutical compositions containing, for
example, an antibody reactive with a mammalian mts-1
20 polypeptide, an antisense mts-1 nucleic acid or the mts-1
1 protein. The mts-1 protein is known to bind calcium
and has a role in the growth of cells (Linzer, *et al.*,
Proc. Natl. Acad. Sci. USA 80:4271-4275, 1983; Jackson-
Grusby, *et al.*, Nuc. Acids. Res. 15:6677-6689; Goto *et*
25 *al.*, J. Biochem. 103:48-53, 1988). The mts-1 protein is
also very closely related to 42A, a gene thought to have
a role in nerve cell growth (Masiakowski, *et al.* Proc.
Natl. Acad. Sci. USA 85:1277-1281, 1988). The mts-1
protein may also have a role in the differentiation of
30 myoepithelial cells (Barracclough, *et al.*, J. Mol. Biol.
198:13-20, 1987). Hence the human mts-1 protein may be
clinically useful, for example, in stimulating cells in

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1 general or preferably, nerve cells, to grow, and
further, in promoting the differentiation of
myoepithelial cells.

5 The active ingredients of a pharmaceutical
composition containing the mts-1 protein or anti-mts-1
antibodies and antisense mts-1 nucleic acids (i.e. anti-
cancer reagents) are contemplated to exhibit effective
therapeutic activity, for example, in promoting cell
10 growth, or for treating cancer, respectively. Thus the
active ingredients of the therapeutic compositions
containing mts-1 protein cell proliferative activity or
anti-cancer reagents, are administered in therapeutic
amounts which depend on the particular disease. For
15 example, from about 0.5 μ g to about 2000 mg per kilogram
of body weight per day may be administered. The dosage
regimen may be adjusted to provide the optimum
therapeutic response. For example, several divided
doses may be administered daily or the dose may be
20 proportionally reduced as indicated by the exigencies of
the therapeutic situation. A decided practical
advantage is that the active compound may be
administered in a convenient manner such as by the oral,
intravenous (where water soluble), intramuscular,
subcutaneous, intranasal, intradermal or suppository
25 routes. Depending on the route of administration, the
active ingredients which comprise mts-1 proteins or
anti-cancer reagents may be required to be coated in a
material to protect said ingredients from the action of
enzymes, acids and other natural conditions which may
30 inactivate said ingredients. For example, the low
lipophilicity of mts-1 protein, and some anti-cancer
reagents, may allow them to be destroyed in the

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1 gastrointestinal tract by enzymes capable of cleaving
peptide or nucleotide bonds and in the stomach by acid
hydrolysis. In order to administer mts-1 protein or
anti-cancer reagents by other than parenteral
5 administration, they should be coated by, or
administered with, a material to prevent its
inactivation. For example, mts-1 protein or anti-cancer
reagents may be administered in an adjuvant, co-
administered with enzyme inhibitors or in liposomes.
10 Adjuvants contemplated herein include resorcinols, non-
ionic surfactants such as polyoxyethylene oleyl ether
and n-hexadecyl polyethylene ether. Enzyme inhibitors
include pancreatic trypsin inhibitor,
diisopropylfluorophosphate (DFP) and trasylol.
15 Liposomes include water-in-oil-in-water P40 emulsions as
well as conventional liposomes.

The active compounds may also be administered
parenterally or intraperitoneally. Dispersions can also
be prepared in glycerol, liquid polyethylene glycols,
20 and mixtures thereof, and in oils. Under ordinary
conditions of storage and use, these preparations
contain a preservative to prevent the growth of
microorganisms.

The pharmaceutical forms suitable for
25 injectable use include sterile aqueous solutions (where
water soluble) or dispersions and sterile powders for
the extemporaneous preparation of sterile injectable
solutions or dispersion. In all cases the form must be
sterile and must be fluid to the extent that easy
30 syringability exists. It must be stable under the
conditions of manufacture and storage and must be
preserved against the contaminating action of

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1 microorganisms such as bacteria and fungi. The carrier
can be a solvent or dispersion medium containing, for
example, water, ethanol, polyol (for example, glycerol,
5 propylene glycol, liquid polyethylene glycol, and the
like), suitable mixtures thereof and vegetable oils.
The proper fluidity can be maintained, for example, by
the use of a coating such as lecithin, by the
maintenance of the required particle size in the case of
dispersion and by the use of surfactants. The
10 preventions of the action of microorganisms can be
brought about by various antibacterial and antifungal
agents, for example, parabens, chlorobutanol, phenol,
sorbic acid, thimerosal, and the like. In many cases it
will be preferable to include isotonic agents, for
15 example, sugars or sodium chloride. Prolonged
absorption of the injectable compositions can be brought
about by the use in the compositions of agents delaying
absorption, for example, aluminum monostearate and
gelatin.

20 Sterile injectable solutions are prepared by
incorporating the active compounds in the required
amount in the appropriate solvent with various of the
other ingredients enumerated above, as required,
followed by filtered sterilization. Generally,
25 dispersions are prepared by incorporating the various
sterilized active ingredients into a sterile vehicle
which contains the basic dispersion medium and the
required other ingredients from those enumerated above.
In the case of sterile powders for the preparation of
30 sterile injectable solutions, the preferred methods of
preparation are vacuum-drying and the freeze-drying
technique which yield a powder of the active ingredient

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1 plus any additional desired ingredient from previously
sterile-filtered solution thereof.

When the mts-1 protein or anti-cancer reagents
are suitably protected as described above, the active
5 compound may be orally administered, for example, with
an inert diluent or with an assimilable edible carrier,
or it may be enclosed in hard or soft shell gelatin
capsule, or it may be compressed into tablets, or it may
be incorporated directly with the food of the diet. For
10 oral therapeutic administration, the active compound may
be incorporated with excipients and used in the form of
ingestible tablets, buccal tablets, troches, capsules,
elixirs, suspensions, syrups, wafers, and the like.
Compositions or preparations according to the present
15 invention are prepared so that an oral dosage unit form
contains between about 0.5 μg and 2000 μg of active
compound.

The tablets, troches, pills, capsules, and the
like, as described above, may also contain the
20 following: a binder such as gum tragacanth, acacia,
corn starch or gelatin; excipients such as dicalcium
phosphate; a disintegrating agent such as corn starch,
potato starch, alginic acid, and the like; a lubricant
such as magnesium stearate; and a sweetening agent such
25 as sucrose, lactose or saccharin may be added or a
flavoring agent such as peppermint, oil or wintergreen
or cherry flavoring. When the dosage unit form is a
capsule, it may contain, in addition to materials of the
above type, a liquid carrier. Various other materials
30 may be present as coatings or to otherwise modify the
physical form of the dosage unit. For instance,
tablets, pills or capsules may be coated with shellac,

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1 sugar or both. A syrup or elixir may contain the active
compound, sucrose as a sweetening agent, methyl and
propylparabens as preservatives, a dye and flavoring
such as cherry or orange flavor. Of course, any
5 material used in preparing any dosage unit form should
be pharmaceutically pure and substantially non-toxic in
the amounts employed. In addition, the active compound
may be incorporated into sustained-release preparations
and formulations.

10 It is especially advantageous to formulate
parenteral compositions in dosage unit form for ease of
administration and uniformity of dosage. Dosage unit
form as used herein refers to physically discrete units
15 suited as unitary dosages for the mammalian subjects to
be treated; each unit containing a predetermined
quantity of the active material calculated to produce
the desired therapeutic effect in association with the
required pharmaceutical carrier. The specification for
the novel dosage unit forms of the invention are
20 dictated by and directly depending on (a) the unique
characteristics of the active material and the
particular therapeutic effect to be achieved, and (b)
the limitations inherent in the art of compounding such
as active material for the treatment of disease in
25 living subjects having a diseased condition in which
bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded
for convenient and effective administration in effective
amounts with a suitable pharmaceutically acceptable
30 carrier in dosage unit form as hereinbefore disclosed.
A unit dosage form can, for example, contain the
principal active compound in amounts ranging from 0.5 μ g

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1 to about 2000 μ g. Expressed in proportions, the active
compound is generally present in from about 10 μ g to
about 2000 mg/ml of carrier. In the case of
compositions containing supplementary active
5 ingredients, the dosages are determined by reference to
the usual dose and manner of administration of the said
ingredients.

As used herein "pharmaceutically acceptable
carrier" includes any and all solvents, dispersion
10 media, coatings, antibacterial and antifungal agents,
isotonic and adsorption delaying agents, and the like.
The use of such media gents for pharmaceutical active
substances is well known in the art. Except insofar as
any conventional media or agent is incompatible with the
15 active ingredient, use thereof in the therapeutic
compositions is contemplated. Supplementary active
ingredients can also be incorporated into the
compositions.

Another embodiment of the present invention
20 relates to the animal tumors and tumor cell lines
developed in accordance with the present invention which
are useful as model systems of the metastatic process.
These tumors and cell lines can be utilized for
screening anti-metastatic drugs and for developing
25 therapeutic regimens for the treatment of malignant
cancer is provided by the present invention. The tumors
provided by the present invention include the IR6 and
IR4 tumors. The tumor cell lines provided by the
present invention include CSML-0, CSML-50, CSML-100,
30 HMC-0, HMC-Lr, T9, T36, LMEC, PCC4c-P, PCC4c-B, PCC4c-
107, IR6CL,, IR4 CL, ELCL,, TRCL, and the murine lung
carcinoma Line 1.

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1 The tumors or cell lines of the present
invention each have a highly predictable metastatic
potential; however the metastatic potentials of related,
but separate, tumors or cell lines can be very
5 different. The properties, and metastatic potentials,
of the tumors and cell lines of the present invention
are fully described in Examples 1, 2, 3 and 12 and in
Tables 1 and 2. While these tumors and cell lines were
derived from mouse mammary carcinomas as well as rat
10 thyroid and epithelial carcinomas, they are useful for
the development of a variety of human cancer therapies,
for several reasons. First, cancer cells all have
similar properties, including, for example, unrestrained
growth and lack of contact inhibition, which suggests
15 that the process of cancer development is similar in all
cancers. Second, the morphologies and biochemical
properties of the tumors developed after injection of
these tumor-derived cells are identical to analogous
tumors in humans. Hence, potential anti-cancer
20 therapies or drugs may effectively be screened by
employing the animal model system of the current
invention.

 The utility of these unique tumors and cell
lines is apparent to one skilled in the art. Briefly,
25 animals are injected with tumors or tumor-derived cells
which have a predictable metastatic potential. A
proportion of the animals are treated with a potential
anti-cancer drug or therapy. After a suitable period of
time, all animals are sacrificed and the tissues of both
30 treated and non-treated animals are examined for the
development of primary and secondary (metastatic)
tumors. If a therapeutic regimen is successful, the

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1 treated animals should have a much lower incidence of
tumor formation.

Both mouse and rat model systems are provided
by the present invention for the development of cancer
5 therapy. A spontaneous mouse mammary carcinoma has been
used to generate different cell lines with low,
intermediate and high incidences of metastasis. This is
done by intramuscular transplantation or subcutaneous
tail transplantations of the original spontaneous
10 mammary tumor cells into syngeneic mice. Intramuscular
transplantation has yielded a cell line called CSML-0
which has low metastatic potential. Solitary lung
metastasis are detected in less than 10% of CSML-0
injected animals sacrificed because of a moribund
15 condition. The highly metastatic CSML-100 cell line has
been generated by selection of the metastatic phenotype
through successive subcutaneous transplantations of CSML
metastatic cells into the tail. The CSML-50 cell line,
selected during the generation of CSML-100, has an
20 intermediate level of metastatic potential.

A variety of rat tumors have been generated by
irradiating normal Fischer 344 rat thyroid cell
suspensions and then transplanting these cells into
rats. Grafts of non-irradiated thyroid cells develop
25 into morphologically and functionally normal thyroid
tissue after transplantation into Fischer 344 syngenic
rats, if elevated levels of thyroid stimulating hormone
are also provided. Irradiation of thyroid cell
suspensions before transplantation has produced a series
30 of rat thyroid carcinomas which are histopathologically
identical to human counterparts. For example, the IR6
tumor, generated in accordance with the present

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1 invention, is highly metastatic, while the IR4 tumor has
low metastatic potential. Both tumors are structurally
and histologically identical to corresponding human
tumors (Fig. 7).

5 The extensive variety of tumors and cell
lines, and the varying metastatic potential of these
tumors and cell lines, provides mouse and rat model
systems amenable to carefully controlled studies
10 directed towards the dissection of the metastatic
process. Therapeutic regimens for treatment of
malignant cancer can be developed by controlled studies
of groups of animals injected with cells of high, low
and intermediate metastatic potential. A drug, or
15 pharmaceutical composition suspected of having anti-
metastatic potential, may be used to treat a proportion
of animals from each group. The incidence of metastasis
amongst the animals receiving the drug or pharmaceutical
composition may be compared with the incidence amongst
20 animals not receiving treatment. Therefore, the present
invention provides an animal system for distinguishing
effective anti-metastatic drugs and therapies from those
that are ineffective.

The Examples serve to further illustrate the
invention without in any way limiting same.

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EXAMPLE 1Materials And Methods1. Medium

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Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) was used for all cell lines. Cells were passed weekly.

2. Metastatic activity

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Metastatic activity was determined by intramuscular injection of 1×10^4 to 1×10^6 tumor cells per tumor cell line in 10-15 mice. Either A/Sn or A/J mice were used.

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For A/Sn mice, cultured tumor cells were trypsinized, rinsed and suspended in sterile Hanks' salt solution. A total of 1×10^6 cells in 0.3 ml of Hanks' solution was injected subcutaneously into each 8 to 10 week old A/Sn mouse. The mice were killed 4-5 weeks after tumor inoculation and the number of lung metastasis was counted. Non-metastatic cell lines were defined as cell lines that did not result in visible metastases. Highly metastatic lines under the same conditions gave rise to multiple metastases in target organs of each mouse.

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Female A/J mice (4-6 weeks old) were injected either with 1×10^4 cells intravenously through the tail vein or with 1×10^6 cells subcutaneously into the abdomen. Fifteen days following intravenous injection and 4-6 weeks after subcutaneous injection, the animals were sacrificed and the lung metastases were counted.

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3. Mouse Tumor Cell Lines

CSML-0, CSML-50 and CSML-100 tumor cell lines were established in accordance with the present

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1 invention from spontaneous mammary adenocarcinomas of
A/Sn mice. These cell lines are described in more
detail in Example 2.

5 HMC-0 and HMV-Lr are tumor cell lines which
were also established from spontaneous mammary
adenocarcinomas of A/Sn mice. T-9, as well as T-36 and
its variant LMEC, are coupled sublines of two original
tumors which were induced by ectopic transplantation of
6-7 day-old gestation syngeneic embryos to CBA/J and
10 A/Sn mice.

Cell lines, PCC4c-P, PCC4,-B and PCC4c-107
were derived from PCC4-Blangy, PCC4-Pasteur and PCC4-107
teratocarcinomas, respectively.

15 A murine lung carcinoma, Line 1, cell line is
highly metastatic, however when Line 1 cells are grown
in the presence of the 3% DMSO, these cells lose their
metastatic potential.

Some of the properties of the above cell
lines, and their metastatic potential, are described in
20 Table 1.

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Table 1

**Metastatic Potential of Analyzed
Mouse Tumors and Mouse Tumor Cell Lines**

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	Tumors and Cell Lines ^a	Spontaneous Metastases	Target Organs
	Mammary carcinosarcoma		
	CSHL-0	low metastatic ^b	lung
10	CSHL-50	50%	lung
	CSHL-100	high metastatic ^c	lung
	Mammary Solid Carcinoma		
	HMC-0	low metastatic	liver ^d
	HMC-Lr	high metastatic	liver ^d
15	Teratocarcinoma cell line		
	PCC4 _u -B	nonmetastatic	-----
	PCC4 _u -P	nonmetastatic	-----
	PCC4 _u -107	nonmetastatic	-----
	C12-	nonmetastatic	-----
	Embryocarcinoma, T-36 node	50%	lymph
20	Cell line derived from T-36, T-36 _u node	50%	lymph
	Embryocarcinoma, LMEV node	high metastatic	lymph
	Teratocarcinoma, T-9 node	low metastatic	lymph
	Colon Adenocarcinoma, Acatol	nonmetastatic	-----
	Melanoma, B-16	low metastatic	lung
	Lung carcinoma, RL-67	high metastatic	lung ^d
	Lewis lung carcinoma, LLC	high metastatic	lung
25	Murine lung carcinoma cell Line 1:		
	Grown without DMSO	high metastatic	
	Grown with 3% DMSO	nonmetastatic	

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^a PCC4_u-B, PCC4_u-P, and PCC4_u-107 are cell lines derived from PCC4-Blangy, PCC4-Pasteur, and PCC4-107 teratocarcinomas.

^b Low metastatic indicates 20% of injected mice give rise to solitary metastases.

^c High metastatic indicates 100% of multiple metastases in target organs.

^d Metastases in other organs.

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1 3. Rat Tumors and Rat Tumor Cell Lines

 An established epithelial cell line, FRTL5, was derived from a culture of rat thyroid cells and is not tumorigenic. In accordance with the present
5 invention, two tumorigenic but non-metastatic derivatives of FRTL5 cells, ELCL, and TRCL,, have also been isolated. The properties of these non-metastatic cell lines are further elaborated upon in Table 2 and in Example 3.

10 The IR6 tumor is a radiation induced, transplantable anaplastic thyroid carcinoma, of epithelial origin. It is a poorly differentiated, highly aggressive adenocarcinoma which is highly metastatic. IR4 is another transplantable, radiation
15 induced thyroid tumor which is moderately differentiated and has low metastatic potential. . The properties of these tumors are further elaborated on in Example 3 and in Table 2.

 4. Nucleic Acid Purification and Analysis

20 Tumor cells were cultivated and prepared for subcutaneous injection into mice as described under the metastatic activity subsection of this section. Injected mice were examined weekly for the appearance of tumors. Tumors were excised and used for DNA and RNA
25 preparations. Total DNA was prepared from cells according to Sambrook et al. (Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, Vol. 2, Laboratory Press, 1989. Pages 9.1-9.62).

 RNAs were prepared from different tumor cells
30 and normal cells according to the procedure described by Chomczynski et al. (1987, Anal. Biochem. 162: 156-159) or Sambrook et al. (Molecular Cloning: A Laboratory

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- 1 Manual, Vol. 1, Cold Spring Harbor Press, 1989:7.1-
7.87). Gel electrophoresis of RNA, RNA blotting to
nylon membrane filters, and hybridization with nick-
translated DNA probes was as described in Grigorian et
5 al. (1985, EMBO J. 4: 2209-2215).

Southern blots were performed using 10 μ g of
genomic DNA extracted from mouse liver, CSML-100 cells,
human placenta and liver, rat liver, pig liver, and
chicken liver. DNAs were digested with BamHI, EcoRI,
10 and PstI endonucleases. Following electrophoresis in a
0.8% agarose gel, the DNA was transferred onto a nylon
membrane (Hybond N, Amersham). The filter was
prehybridized and hybridized following the standard
procedure of Sambrook et al., supra.

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EXAMPLE 2Development of Benign and
Metastatic Mouse Tumor Cell Lines

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CSML-0, CSML-50 and CSML-100 are tumor lines established from spontaneous mammary adenocarcinomas of A/Sn mice. CSML-0 was derived from a tumor maintained by intramuscular passages and was characterized as having a low metastatic potential. Solitary lung metastases were detected in less than 10% of autopsied animals that had been killed because of a moribund condition. A second, highly metastatic subline, CSML-100, was developed by selecting for a metastatic phenotype in successive transplantations (via successive subcutaneous tail injections) of initially rare, and subsequently more frequent, CSML metastatic tumor cells. The frequency of metastasis to the lung by CSML-100 cells was 100%, by any route of primary inoculation. CSML-50 represents a cell line with an intermediate level of metastatic potential which was developed during the establishment of CSML-100. The frequency of lung metastasis by CSML-50 cells was about 50%.

The CSML-100 tumor line also caused tumors to form in A/J mice (Jackson laboratories) which have a similar genotype to that of A/Sn mice. CSML cells were not rejected by A/J mice and metastases were detected in lungs and other organs by any injection route.

A/J mice intravenously injected with CSML-100 developed tumors within 6-7 days of injection. Even when only 1×10^4 CSML-100 cells were injected, abundant metastases were found in lungs by 15 days post-injection

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1 (Fig. 9c). A/J mice injected with CSML-100 cells by the
subcutaneous route had approximately 250 spontaneous
metastases per lung 4-6 weeks later (Fig. 9d). Mice
5 injected with CSML-0 by either route of injection had
only 10-25 tumors per lung (Fig. 9a and 9b). After
sacrifice of each mouse, the ovaries, liver, kidney,
gonads, muscle, and brain tissues were preserved for
immunohistochemical analysis. Such analysis indicated
10 mts-1 was highly expressed in metastasized tumors,
particularly in the ovarian and lung tumors.

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EXAMPLE 3Development of Benign and
Metastatic Rat Tumors and Rat Tumor Cell Lines

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A number of rat thyroid carcinomas and cell lines have been developed in conjunction with the present invention, by irradiating normal Fischer 344 rat thyroid cell suspensions before transplantation into the rat. Grafts of non-irradiated, monodispersed rat thyroid cells develop into morphologically and functionally normal thyroid tissue within a short time after transplantation into Fischer 344 syngeneic rats, if the level of thyroid stimulating hormone (TSH) within the rat is elevated by injection of TSH. If thyroid cells are irradiated before transplantation, thyroid carcinomas develop. The IR6 tumor was obtained as a radiation induced, transplantable anaplastic thyroid carcinoma of epithelial origin. IR6 was found to be poorly differentiated, highly metastatic and did not require TSH for growth. The IR4 tumor was also obtained as a radiation induced rat thyroid carcinoma but IR4 is moderately differentiated into a follicular carcinoma, grows slowly only when TSH is provided and has low metastatic potential. IR6CL, is a cell line derived from the IR6 tumor which retains the original properties of the parent IR6 tumor, e.g., it grows independently of TSH, is poorly differentiated and is highly metastatic.

An established epithelial cell line, FRTL5, derived from a culture of rat thyroid cells was also obtained. FRTL5 cells requires TSH and remains highly differentiated, but produces no tumors when injected

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1 subcutaneously into syngeneic Fischer 344 rats. Two
 2 tumorigenic derivatives of the FRTL5 cell line, ELCL,
 3 and TRCL,, have also been isolated and characterized.
 4 ELCL, was obtained as a spontaneous mutant of FRTL5, and
 5 subsequently established as a transformed cell line
 6 which required low levels of TSH for growth. ELCL,
 7 formed primary tumors upon subcutaneous injection in
 8 syngenic rats but no metastasis was observed. TRCL, was
 9 a radiation induced mutant of FRTL, which was then
 10 established as a transformed cell line with no TSH
 11 requirement for growth. TRCL, cells produced fast-
 12 growing primary tumors with little or no potential for
 13 metastasis.

14 Some of the properties of the above described
 15 tumors and cell lines are summarized in Table 2.

Table 2

Metastatic Potential of Rat
 Tumors and Rat Tumor Cell Lines

Tumors and Cell Lines	Spontaneous Metastases	Target Organs
25 Thyroid carcinoma IR6 tumor IR4 tumor	high metastatic low metastatic	Lung, Liver, Kidney
Thyroid cell line FRTL5 (non-tumorigenic) ELCL, (tumorigenic) 30 TRCL, (tumorigenic)	nonmetastatic nonmetastatic nonmetastatic	

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EXAMPLE 4Isolation of the Murine mts-1 Gene

5 mRNA from CSML-100 and CSML-0 cell lines was prepared as described by Chomczynski et al. supra, and polyadenylated mRNA was selected as in Sambrook et al. (Molecular Cloning: A Laboratory Manual, Vol. 1, Cold Spring Harbor Laboratory Press, 1989. Pages 7.1-7.29).
10 2µg poly (A)⁺ mRNA from highly metastatic CMSL-100 cells was treated with reverse transcriptase under conditions appropriate to generate a single stranded complementary DNA (cDNA) (Sambrook et al., supra. Vol. 2. Pages 8.1-8.86). This CMSL-100 cDNA pool was subjected to
15 subtractive hybridization with 50µg poly (A)⁺ mRNA from low metastatic potential CMSL-0 cells to remove cDNA's with no role in the development of metastasis. The cDNA/RNA mixture was heated at 100°C for 5 min., cooled on ice and placed in a final reaction volume of 1 ml in
20 7% phenol (adjusted to pH 7.6 with 0.1M Tris-HCl, 1.25M NaCl, 120 mM sodium phosphate buffer, pH6.8) in a 10 ml glass centrifuge tube in. The tube was shaken for 7 days at 25°C. After hybridization, the mixture was extracted twice with chloroform, dialyzed against 10 mM
25 Tris-HCl (pH 7.5), 1 mM EDTA to remove excess salts, and then precipitated with ethanol. Double stranded cDNA/mRNA, representing functions which are not unique to the metastatic phenotype, were removed by passage through a hydroxyapatite column. The single stranded
30 cDNA was made double stranded and cloned into a λgt10 vector by standard procedures (Sambrook et al., supra pages 8.1-8.86).

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1 Functions expressed highly during metastasis
were detected by differential hybridization with CSML-
100 and CSML-0 P-labeled cDNA probes. Mouse mts-1 cDNA
5 clones were identified as strongly hybridizing with the
DCSM-100 probe but weakly hybridizing with the CSML-0
probe.

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EXAMPLE 5Isolation of a Rat mts-1 cDNA

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Rat cDNA libraries were prepared from normal thyroid and radiation induced thyroid carcinoma tissues as well as cell lines derived from normal and carcinogenic thyroid tumor cells. Poly (A)⁺ mRNA was purified from highly metastatic IR6 tumors and from low metastatic potential IR4 tumors. Single-stranded cDNA was synthesized from IR6 poly (A)⁺ RNA and the IR6 mRNA was hydrolyzed. This IR6 cDNA pool was subjected to subtractive hybridization with a 50-fold excess of IR-4 poly (A)⁺ mRNA according to the phenol emulsion reassociation technique (PERT method) of Kohne *et al.* (1977, *Biochemistry* 16: 5329-5341). Single stranded cDNA, representing functions likely to be involved in the metastatic phenotype, was isolated from the subtractive hybridization mixture by passage through a hydroxyapatite column (which will bind double stranded nucleic acids, i.e. the RNA:DNA hybrids representing the IR6 functions of low metastatic potential) followed by alkaline hydrolysis of the remaining IR4 mRNA. The single-stranded cDNA pool was made double stranded and cloned into a λ gt10 cloning vector.

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The subtracted IR6 cDNA library was screened differentially with ³²P-labeled single stranded cDNA probes generated by treatment of IR6 and IR4 poly (A)⁺ mRNA with reverse transcriptase. mts-1 clones were identified by strong hybridization with the IR6 probe but weak hybridization with the IR-4 probe.

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EXAMPLE 6Isolation of the Human mts-1 cDNA

5 A human cDNA library was constructed in λ gt10 using poly (A)⁺ RNA prepared from HeLa cells. The library was screened with a ³²P-labelled mouse mts-1 cDNA probe at 42° in 50% formamide. Filters were washed in 2 x SSC with 0.1% SDS at room temperature and then
10 twice in 0.2 x SSC with 0.1% SDS at 50°C. Strongly hybridizing cDNA clones were sequenced; the human mts-1 cDNA was identified by high sequence similarity to the mouse mts-1 cDNA in regions outside the highly conserved Ca⁺⁺ binding domain. This human mts-1 clone is full
15 length as judged by sequencing of the human genomic mts-1 gene and by primer extension analysis of mts-1 mRNA using mts-1 oligonucleotide probes. The nucleotide and amino acid sequences of the human mts-1 gene are provided as SEQ ID NO: 1 and 2, and also given in Fig. 1
20 and 2.

 The mts-1 cDNA was also isolated from human melanoma cell line Wm64 by reverse transcription of mRNA isolated from those cells followed by polymerase chain reaction.

25 Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

 Total RNA from human melanoma cell line Wm64 was pretreated with RNase free DNase I (1U/ μ l) in 2 mM MgCl₂ for 30 minutes at 37°C then 95°C for 5 minutes to inactivate the DNase; poly A⁺ RNA was not routinely
30 treated with DNase I before an RT-PCR experiment. RNA (1 μ g total RNA or 50 ng poly A⁺ RNA) was reverse transcribed in the presence of 50 mM Tris-HCl pH 8.3,

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1 75 mM KCl, 3 mM MgCl₂, 3 μM oligo-dT₁₈, 0.3 mM each
dNTP, 200U M-MLV reverse transcriptase at 22°C for 10
minutes, 42°C for one hour and 90°C for 10 minutes. The
following human mts-1 primers were used for synthesis of
5 the human mts-1 cDNA by PCR reaction:

5' ATG GCG TGC CCT CTG GAG AAG - 3' (SEQ ID NO:8)

5' TTT CTT CCT GGG CTG CTT ATG - 3' (SEQ ID NO:9).

PCR amplification was done in 10 mM Tris HCl (pH 8.4),
50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin with 2.5 mM dNTP,
10 0.6 μM each primer and 1.25U Taq DNA polymerase.
Amplification was by 35 cycles of: 94°C for 1 min; 52°C
for 2 min; 72°C for 3 min, followed by a 7 min extension
period at 72°C.

Amplified DNA was isolated from a 1% agarose
15 gel and cloned into a baculovirus transfer vector as
described in Example 7.

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EXAMPLE 7Expression of the mts-1 Gene Product

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Overexpression of the mts gene product, is accomplished by DNA transfections using the vector system described by Lockshon and Weintraub. This vector is a pUC19 based vector system, very similar to the Bluescript™ vector (Fig. 3). In the unique HindII site of the Bluescript™ vector, a eukaryotic control element harboring a strong murine sarcoma virus promoter, followed by a unique EcoRI site, followed by SV40 polyadenylation sequences is introduced. The complete mts-1 cDNA is introduced into the unique EcoRI site downstream from the MSV-LTR sequences. Because of the presence of an internal EcoRI site in the mts-1 cDNA, partial EcoRI digestion of the mts-1 recombinant is done to isolate the entire mts-1 cDNA molecule. Retroviral promoters with LTRs are very strong and overexpression of the mts transcript is expected. The mts-1 recombinant expression vector can be used for both permanent or transient expression. However, stable (permanent) transfectants are desirable because stable transfectants can be clonally purified, and represent a homogeneous population of a given phenotype useful for quantitating metastatic potential.

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Expression of mts-1 protein from a pTrcHisB vector:

Large quantities of the mts-1 specific protein were expressed using the inducible bacterial vector pTrcHisB (Invitrogen) (Fig. 10a). Murine mts-1 cDNA was subcloned in frame (confirmed by sequence analysis) into a BamHI-KpnI site with the multiple cloning site of

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1 pTrcHisB. This generated plasmid pTBM1. The fusion
protein expressed by pTBM1 had 6 tandem histidine
residues (which have a high affinity for a Ni^{++} charged
resin), an enterokinase specific cleavage site, and the
5 mts-1 protein product. Expression of the fusion protein
encoded by pTBM1 was induced by IPTG. Similar
constructs were generated with human mts-1 cDNA.

Expression of mts-1 protein in a baculovirus expression
vector:

10 A plasmid containing the cytomegalovirus
promotor was used to construct pCMV/mts-1, or
pCMV/mts-1, high expression vectors harboring mts-1
human and murine cDNAs, respectively.

The baculovirus expression vector mts-1-
15 BacPAK₆ plasmid was constructed from the pCMV clones as
follows. pCMV-mts-1 was digested with BamH1, and the
mts-1 cDNA fragment was purified from a 1% agarose gel.
The purified fragment was ligated into BamH1-cleaved
pBacPAK1 and the ligation mix was transformed into E.
20 coli JM109 cells. Positive clones were identified and
plasmid DNA was sequenced to confirm the orientation and
integrity of the ligation junction.

Transfer vector pBacPAK-mts-1 was transfected
into Sf21 cells, along with Bsu361 digested BacPAK6
25 viral DNA. Soon after infection, the cells were
overlayered with 1% agarose to visualize the plaques and
to prevent mixing of clones. After 4-5 days of
infection, the cells were stained with neutral red which
is taken up by healthy cells, but not by the dead cells.
30 Plaques appeared as clear circles against red or pink
background.

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1 Western Blot analysis using the α -mts-1
antibody was conducted to confirm that several mts-1
recombinant viruses produced mts-1 proteins.

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EXAMPLE 8Purification of mts-1 Protein

5 Purification of the mts-1 protein parallels
that of other S100 family members which have been
purified to homogeneity from bovine brain (Baudler, et
al. J. Biol. Chem. 261: 8204-8212, 1986). Exceedingly
high degrees of purification can be achieved because of
10 the stability of the protein and the availability of
several affinity chromatography steps including
phenothiazine-agarose, zinc dependent binding to phenyl
sepharose. FPLC chromatography on Mono Q is known to
separate S100 family members and other HPLC columns have
15 been developed such as melittin silica, to affinity
purify S100 proteins. Tissues or cells providing large
amounts of mts-1 include not only the bacterial, yeast
and mammalian cell lines engineered to express large
quantities of recombinant mts-1, but also the highly
20 metastatic tumors and cell lines shown to express mts-1
by the present invention.

Purification of His-mts-1 Fusion

 An overnight culture transformed with pTBM1
25 was diluted 1:100 and allowed to grow 1.5 hours (until
OD₆₀₀ = 0.3). The culture was then induced with 1mM
IPTG and allowed to grow 4.5 hours more at 37°C. Cells
were harvested, cell pellets were then collected by
centrifugation and resuspended in a 6M guanidinium-HCl
30 buffer. The cells were stirred for 1 hour and then
centrifuged at 18K rpm for 15 min at 4°C. The
supernatant was collected and added to a 50% slurry of

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1 Ni⁺⁺-NTA resin (obtained from Qiagen). The mixture was
stirred for an hour and loaded onto a column. The
column was washed in a series of urea based buffers
5 pH). The protein was eluted in 3 ml fractions using
buffer D (8M urea, 0.1M Na Phosphate, 0.01M Tris/HCl, pH
5.9). A large amount of the protein did not elute until
the pH of the buffer was lowered to 4.5 (buffer E):
monomeric forms of the histidine fusion eluted in buffer
10 D, whereas aggregates eluted in buffer E. Aliquots of
each fraction were boiled in SDS-PAGE loading buffer and
loaded onto 12% SDS-polyacrylamide gels. The gels were
stained with Coomassie Brilliant Blue. The results of
such experiments are depicted in Fig 10b. After the
15 purity of the His-Mts1 fusion protein was confirmed,
assays were performed to determine relative protein
concentrations. Fractions D2 and E1 (which contained
approximately 3.2 mgs protein total) were pooled and run
on another SDS-polyacrylamide gel. Strips were cut out
20 from the gel and stained in Coomassie Brilliant Blue to
determine the location of the His-Mts1 protein in the
gel. The portion of the gel containing the His-Mts1
fusion was cut out and the protein was isolated from the
gel by elution.

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EXAMPLE 9Generation of Polyclonal Antibodies5 Antibodies Against mts-1 Peptides

Synthetic oligopeptides with the following amino acid sequences were made:

- 1) Human mts-1 amino acids 2-11 (unique):
Ala-Cys-Pro-Leu-Glu-Lys-Ala-Leu-Asp-Val
- 10 2) Human mts-1 amino acids 22-37 (the calcium binding domain):
Lys-Glu-Gly-Asp-Lys-Phe-Lys-Leu-Asn-Lys-Ser-Glu-Leu-Lys
Glu-Leu
- 15 3) Human mts-1 amino acids 42-54 (unique):
Leu-Pro-Ser-Phe-Leu-Gly-Lys-Arg-Thr-Asp-Glu-Ala-Ala
- 4) Human mts-1 amino acids 87-101 (unique):
Asn-Glu-Phe-Phe-Glu-Gly-Phe-Pro-Asp-Lys-Gln-Pro-Arg-
Lys-Lys

Peptides 1, 3 and 4 were chosen as mts-1 antigens because they encode unique proteins of the mts-1 protein, i.e. these regions of the mts-1 protein do not share homology with other proteins, in particular with other calcium binding proteins. Peptide 2 was chosen because it encodes the calcium binding domain of mts-1. Therefore, peptide 2 generates antibodies reactive with many members of the calcium binding protein family.

New Zealand white female rabbits were immunized by subdermal injection with 100 μ l of Freund's complete adjuvant containing 0.1-1 mg of oligopeptide in 10 locations along the back. The rabbits were first shaved on both sides of the back for easy subdermal

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- 1 injection. The antigen-adjuvant mixture was prepared by
mixing in two connected 1 ml glass teflon syringes.
Typically rabbits are then injected with about 1 mg of
antigen at each 2 month interval following the primary
5 injection, until the serum is positive at a dilution of
greater than 10^{-4} when assayed by immunoblotting.

Antibodies Against Whole mts-1 Protein

- The mts-1 protein was expressed as a His-Mts1
10 protein (Examples 7 and 8). Host cell lysates
containing the His-Mts1 protein were fractionated over a
 Ni^{++} -NTA column (Qiagen). Fractions containing the most
His-Mts1 protein were pooled and electrophoresed on an
SDS-polyacrylamide gel. The purified protein was eluted
15 from the gel and sequenced to confirm that it was His-
Mts1.

- Three chickens were then immunized with the
purified His-Mts1 protein. Chickens were chosen for two
reasons. First, mts-1 is highly conserved in mammals
20 and an avian system was expected to provide a better
immune response. Second, antibodies can easily be
obtained from the eggs of the chickens. Continuous
bleeding of the animal to obtain antibodies is,
therefore, avoided.

- 25 The polyclonal antibody generated was named α -
mts-1, and its efficacy on Western blots and tissues was
established (see Example 16 and Figs. 13 and 14).

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EXAMPLE 10Monoclonal Antibody Production

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Monoclonal antibodies are prepared in accordance with the techniques developed by Kohler and Milskin (Eur. J. Immunol. 6:511-519, 1976) and Harlow et al. (Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988). Balb/c mice are immunized subdermally with 100 ul of Freund's complete adjuvant containing 0.1-1 mg of the conjugated or non-conjugated mts-1 oligopeptides described in Example 9. Two weeks after the initial injection, the mice are boosted with the appropriate mts-1 antigen by intravenous and intraperitoneal injection of 100 ug of antigen in phosphate buffered saline (PBS).

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Five days after the last injection and after confirmation of the presence of antibody in mouse sera, the mice are sacrificed and their spleens removed. Spleen cells are obtained by gentle disruption of the spleen in a 7 ml Dounce homogenizer in 3.5-4 ml PBS. The cells are then pelleted at 1200 rpm in a PR6 centrifuge for 6 minutes at room temperature. The supernatant is removed into a suction flask, and the cells are resuspended in 15 ml 0.83% NH_4Cl . This suspension is incubated at room temperature for 5 minutes then underlain with 10 ml fetal calf serum at 37°C. The cells are again pelleted by centrifugation for 8 minutes, at 1200 rpm at room temperature, then the supernatant is withdrawn into a suction flask cells resuspended in 20 ml PBS.

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1 The following solutions are prepared for use
in the subsequent cell fusion:

Hypoxanthine (H), 680 mg/100 ml H₂O; add 204 drops
conc. H₂SO₄; heat to dissolve

5 Aminopterin (A), 46.4 mg/100 ml H₂O; add 2 drops
1.0 N NaOH to dissolve

Thymidine (T), 775 mg/100 ml H₂O; add 45 mg glycine
PEG-DME--melt PEG at 42°C, then add 1 ml DME (at
37°C); adjust pH with 1.0 N NaOH to 7.6

10 DMEM--to 500 ml DME add 37.5 ml a- horse serum;
37.5 ml FCS, 10.0 ml L-glutamine, 0.2 ml garamycin

2X HAT-DME--to 200 ml DME add 25.0 ml a- horse
serum, 25.0 ml FCS, 4.0 ml L-glutamine, 0.2 ml
garamycin, 0.8 ml H, and 0.8 ml A, and 0.8 ml T (2X HT-
15 DME omits A)

Cloning Agar--350 mg unwashed Difco agar in 25 ml
H₂O, autoclaved

Cloning Medium--to 25 ml 2X DME, add 35 ml
filtered, condition DMEM, 7 ml a- horse serum, 7 ml
20 FCS, 1ml L-glutamine, .1 ml garamycin.

Two 30 ml flasks of plasmacytoma P3 NS1/1-Ag4-
1 cells are added to centrifuge tubes and spun down at
1200 rpm for 8 minutes at room temperature. The spleen
cells are resuspended in 20 ml PBS. From each
25 suspension, .01 ml is removed and added to 0.1 ml 0.4%
trypan blue and 0.3 ml PBS and the cells counted. The
volume of each suspension is adjusted so as to obtain a
spleen cell to NS1/1-Ag4-1 cell ratio of 10:1, and the
suspensions are then mixed. The mixture is pelleted at
30 1200 rpm for 8 minutes at room temperature and all but
about 0.1 ml of supernatant removed. The cells are then
resuspended in the remaining liquid and then added to

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1 1.3 ml of 1:1 PEG-DME solution, pH 7.6. Every minute
the volume of the solution is doubled with DME until the
final volume is 25 ml.

5 The cells are again pelleted, the supernatant
decanted, and the cells resuspended in enough 50% 2X
HAT-DME/50% conditioned DMEM (the supernatant retained
form the Sp2/0 cells above) to yield a final
concentration of about 3.5×10^6 spleen cells. The
cells are distributed into a 96-well flat-bottom
10 microtiter plate (TC-96; Flow Laboratories), at 0.1
ml/well. The plate is incubated at 37°C in humidified
air/CO₂ until visible colonies appear, usually about 10-
12 days. The contents of the well is transferred to 0.5
ml of HAT-DME/conditioned DME in a TC-24 plate (Flow
15 Laboratories). When healthy cell growth appears (about
2-5 days), about .35 ml medium is removed and tested for
antibody production by enzyme-linked immunosorbent assay
(ELISA), hemagglutinin inhibition assay, or
neuraminidase inhibition assay. When those cells
20 producing the antibodies of interest are growing well,
one drop for each culture is transferred into 1.0 ml
DMEM in a TC-24.

To clone the hybrid cells, 25 ml of melted
agar and 76 ml of cloning medium is combined, and 5 ml
25 is pipetted into 60 mm petri dish and left to
solidify. Cells from DMEM cultures are diluted in 50%
DMEM/50% conditioned DMEM, 10^{-1} or 10^{-2} depending on cell
growth. Into sterile tubes is placed 0.1 ml of each of
the two dilutions, and to each is added 0.9 ml of
30 cloning medium/agar mixture. This is mixed well and
poured over the surface of the agar underlay. After
solidification the plates are incubated at 37°C

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1 incubator until colonies are visible with the naked eye,
typically about 7-10 days. Colonies are then picked and
transferred .1 ml of DMEM/conditioned DMEM in a TC-99
plate and incubated at 37°C in a CO₂ incubator. After
5 the culture is acidic (usually 1-4 days), transfer is
made to 0.05 ml DMEM in TC-24 plate. When the growth is
50% confluent, the medium is removed and tested for
antibody production are previously. Those clones
producing mts-1 specific antibodies are moved into 5 ml
10 DMEM in 25 cm² flasks. Cloned cells are then frozen or
injected into mice for ascites production.

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EXAMPLE 11Sandwich Assay For mts-1

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For detection of the presence of mts-1 in serum or cleared cell lysates of tissue specimens, approximately 100 ul of a monoclonal antibody prepared as in Example 9 or 10 is immobilized on latex beads and is contacted with about 100 ul of the serum or cleared lysate to be tested. The immobilized antibody and lysate are allowed to react for a period of about ten minutes and then the latex beads with the mts-1 antigen bound to the immobilized antibody are rinsed with a solution of PBS (phosphate buffered saline). To the latex beads is then added about 100 ul of mts-1 specific antibody conjugated to horseradish peroxidase. The labeled antibody bead mixture is incubated for a period of about ten minutes. At this time, an enzyme substrate, hydrogen peroxide and aminoantipyrine, are contacted with the beads, and this mixture is incubated for a period of about 5-10 minutes, at which time the development of color in the sample is an indication of a positive reaction and the presence of mts-1.

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EXAMPLE 12Expression of mts-1 is 10-100 Fold Higher in
Metastatic Tumor Cells Than in Non-Metastatic Cells

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To examine the expression levels of mts-1, mRNA was purified from metastatic and benign tumors, and cell lines derived from such tumors, as well as from corresponding normal tissues. Purified RNA was size fractionated in a gel and blotted onto nylon membranes for Northern analysis with mts-1 nucleic acid probes.

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Fig. 4 shows that the CSML-0 cell line of the present invention, which has a very low metastatic potential, had very low, or non-detectable levels of the mouse mts-1 transcript. In contrast, the CSML-100 cell line of the present invention, which has an extremely high metastatic potential, expressed high levels of mts-1. It is estimated that metastatic CSML-100 cells express at least 100-fold more mts-1 than do non-metastatic CSML-0 cells.

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Similarly, in a separate experiment, various metastatic and non-metastatic tumors and tumor cell lines were tested for their mts-1 expression levels, by Northern analysis using a ³²P-labeled mouse mts-1 probe. The properties of these tumors and cell lines are described in detail in Examples 1, 2 and 3 and in Tables 1 and 2. As shown in Fig. 5, only those tumors and cell lines which are metastatic (indicated by an "M" above the gel lane) exhibit high levels of mts-1 expression. Metastatic cell types exhibiting increased mts-1 expression include: RL-67 lung carcinoma tumors, Lewis

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1 Lung carcinoma tumors, LMEC embryo-carcinoma tumors, and
T-36 embryo-carcinoma tumors and cell lines.

Fig. 6 shows that the highly metastatic
adenocarcinoma rat tumor, IR6 (lane 5), and a cell line
5 derived from IR6 (lane 7), as well as a metastatic cell
line derived from a mouse lung carcinoma, Line 1 (lane
3) all exhibit 10-100 fold increased levels of mts-1
expression compared to a tumorigenic but non-metastatic
cell line, TRCL₁ (lane 6) or a non-tumorigenic FRTL5
10 cell line (lane 8).

Hence these data demonstrate unequivocally
that mts-1 expression is increased 10-100 fold in
metastatic cells of diverse types relative to normal
cells or non-metastatic (benign) tumor cells.

15 Table 3 further illustrates that only
metastatic cells or cells with a high degree of motility
express high levels of mts-1 RNA. Detection was by
northern analysis using γ -actin expression for
normalization. Autoradiograms were densitometrically
20 traced, and a numerical value between 0-5 was assigned
relating the tracing peak height to the amount of
expression. The status of each cell type tested was
characterized as normal (N), benign (B), metastatic (M)
or cell line (C). The number of samples tested is
25 indicated under the status of cell type.

Table 3 illustrates that only metastatic cell
types have an mts-1 expression level greater than 0.5.
Accordingly, high levels of mts-1 expression are
observed in numerous metastatic cell types including,
30 for example, liver hepatomas, lung carcinomas,
pancreatic cancers, breast adenocarcinomas, endometrial
cancers, ovarian cancers, cervical cancers, melanomas,

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1 lymphomas and leukemias. However, such high levels of
2 mts-1 expression are observed only in metastatic cells,
3 non-metastatic cells do not express high levels of
4 mts-1.

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Table 4

Selective Expression of *mts-1* in Metastatic
Cells or Cells with High Degree of Motility

Phenotype of Tissue	No. of Samples; Status				Level of Expn.
	N	B	M	C	
Adult Liver	5	-	-	-	0
Liver Adenoma	-	5	-	-	0
Liver Hepatoblastoma	-	5	-	-	0
Liver Hepatoma	-	-	4	-	1.0
Adult Colon	4	-	-	-	0.4
Colon Carcinoma	-	5	-	-	0.43
Adult Kidney	2	-	-	-	0.1
Kidney Carcinoma	-	2	-	-	0.1
Adult Lung	2	-	-	-	0.1
Small Lung Carcinoma	-	-	2	-	1.0
Adult Pancreas	1	-	-	-	0
Pancreatic Cancer	-	-	1	-	1.0
Normal Breast	4	-	-	-	0
Breast Carcinoma	-	2	-	-	0
Breast Adenosarcoma	-	-	-	2	1.0
Endometrial Cancer	-	-	2	-	1.5
Ovarian Cancer	-	-	2	-	1.4
Cervical Cancer	-	-	2	-	1.5
ASPC 1 Pancreatic Cancer	-	-	-	1M	1.0
AN3CA Endometrial Cancer	-	-	-	1M	1.5
BIX3A Ovarian Cancer	-	-	-	1M	1.5
Hela Cervical Cancer	-	-	-	1M	1.5
MCF7-1 Breast Cancer	-	-	-	1	0
AS49-1 Lung Cancer	-	-	-	1(M)	0.8
MC1 Neuroblastoma Line	-	-	-	1	0
Y79 Retinoblastoma	-	-	-	-	0
Primary Melanoma Wm278	-	-	-	1	0.5
Corcl Primary Melanoma	-	-	-	0	1.0
Wm8 Melanoma	-	-	-	1(M)	2.0
Wm164 Melanoma	-	-	-	1(M)	2.0

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Table 4 (cont.)Normal B Cells Do Not Express mts-1B-Cell Lymphoma & Leukemias

<u>Type</u>	<u>No.</u>	<u>Level of Expn.</u>
Cleaved B Cell Lymphoma	1	3
Hairy Cell Leukemia	1	4
CML Crisis B Cells	3	3

Leukemias

<u>Type</u>	<u>No. of Samples Tested</u>	<u>Average mts-1 Expn. Level</u>
CML (chronic probe)	23	0.49
CML (crisis)	12	1.9
CMML	1	1.0
ALL	1	3.0
AML	6	0.7
AMML	2	1.0
Pure Monocytic Leukemia	3	1.5

Abnormal Blood Infiltrated with High WBC
Count Separated by Ficoll-Hypaque Gradient

Pellet	4	0.3
Interface	5	0.6

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EXAMPLE 13Introduction of the mts-1 Gene into
Cultured Cells Confers a Metastatic Phenotype

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According to the present invention, mts-1 is not expressed in normal, or nonmetastatic tumor cell lines, from the rat thyroid or the mouse lung. However, the highly metastatic Line 1 cell line, derived from a mouse lung carcinoma, does express mts-1 mRNA. When Line 1 cells are grown in the presence of 3% DMSO, they lose their metastatic potential and also do not show detectable levels of mts-1 mRNA. These data indicated that mts-1 expression is correlated with the metastatic phenotype.

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To establish that high levels of mts-1 expression can confer a metastatic phenotype the rat mts-1 cDNA was cloned into the MSV vector depicted in Fig. 3, to allow high expression of the mts-1 protein. This mts-1 expression vector was co-transfected into mouse lung carcinoma Line 1 cells with a plasmid encoding a selectable neomycin (Neo) gene. Stable cell lines resistant to neomycin were tested for integration of the mts-1 gene into their genome by Southern blot analysis of their genomic DNA. The controls for this experiment were Line 1 cells stably transfected only with the selective neomycin resistance gene grown in the presence of 3% DMSO, as well as non-transfected Line 1 cells grown without DMSO.

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Ten transfectants (N1-N10) possessing the transfected mts-1 gene were grown in 3% DMSO to test whether acquisition of the highly expressed recombinant

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1 mts-1 gene could generate a metastatic phenotype in
cells that are normally not metastatic. 10⁷ cells of
transfectants N2, N3, N4, N5, and N8 were injected in
the tail veins of 3 mice. As controls, 10⁷ cells of
5 Line 1 cells, and two neomycin only transfectant cell
lines (Neo 2 and Neo 3) were injected into the tail
veins of 3 mice. The animals were sacrificed after 2
weeks and tested for lung metastasis after staining with
India ink and fixation. The animals injected with N4
10 and N5 cells grown in 3% DMSO prior to injection,
exhibited high levels of metastasis, equivalent to Line
1 cells grown in the absence of DMSO, while other cell
lines gave rise to low levels of metastasis. The fact
that not all transfected cell lines gave rise to high
15 levels of metastasis might have been due to a variation
in mts-1 expression levels caused by mts-1 insertion
into "silent" regions of the genome. To examine the
expression levels of mts-1 in N1-N10 transfectants grown
in 3% DMSO, mRNA was extracted from these cell lines
20 prior to injection into mice, and analyzed for mts-1
mRNA expression levels by Northern analysis. As shown
in Fig. 8, not all transfectants exhibit high levels of
mts-1 expression, probably because of the influence of
genomic regulatory elements lying near the mts-1
25 insertion site. Transfectant cell lines N3, N4 and N5
have high levels of mts-1 expression, but the N3 cell
line gives rise to a low molecular weight mts-1
transcript, indicating that the mts-1 gene of this
transfectant cell line may be defective due to a
30 rearrangement during transfection and integration into
the genome.

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1 Table 3 shows that similar data were obtained
by intravenous injection into rats of transfectant cell
lines containing expression vectors with the rat mts-1
gene in a sense and antisense orientation, relative to
5 the MSV LTR promoter.

Hence, these data indicate that the metastatic
phenotype can be generated in non-metastatic cells by
the introduction of a highly expressed mts-1 gene.

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Table 3

**Mouse Lung Metastasis Counts
Using Different mts-1 Transfectants**

	Neo+	Line I+		Clone 156/3 (N ₃)+	Clone 156/4 (N ₄)+	Clone 156/5 (N ₅)+
	<u>DMSO</u>	<u>DMSO</u>	<u>Line I</u>	<u>DMSO</u>	<u>DMSO</u>	<u>DMSO</u>
Intravenous	5	57	190	342	355	360
Injection	0	38	205	300	495	460
10 ⁶ Cells	0	65	251	320	310	310
Into Tail Vein	11	68	300	75	142	120

rat mts-1 clone 156 = sense construct
 rat mts-1 clone 162 = antisense construct

In the above experiment IR6 tumor cells alone generate lung metastasis in 20% of the injected rats, with 1-2 tumors observed in the kidneys of some rats. 50% of rats injected with transfectants containing mts-1 in a sense orientation (cell lines 156/2, 156/7 and 156/8) had metastases, while 10% of rats injected with transfectants containing mts-1 in an antisense orientation (cell lines 162/9 and 162/1) had metastases.

Hence transfection of a mammalian mts-1 gene into mice or rat cells can cause such cells to undergo metastasis when they are injected into a mouse or rat.

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EXAMPLE 14CSML-100 Cells Grow More Slowly than CSML-0 Cells5 Methods:

CSML-0 and CSML-100 cells were seeded at a density of 10^6 cells/dish and counted the following day using a hemacytometer. The relative rates of DNA synthesis were measured by incorporation of $^3\text{[H]}$ thymidine. Both experiments were done in triplicate, and the data are reported as an average.

DNA synthesis was measured as follows. The cells were washed once with media. 2 mls of media containing 1 μl of $^3\text{[H]}$ thymidine was added to the cells and incubated for 4 hours. The cells were washed twice with PBS and TCA precipitated following standard protocols. The TCA precipitate was dissolved in 0.1N NaOH containing 0.5% Triton X-100 and placed on ice for 30 minutes. The resultant suspension was added to 6 mls of scintillation fluid for scintillation counting.

Results:

Table 5 illustrates that less cell growth and less tritiated thymidine incorporation was observed for metastatic CSML-100 cells than for non-metastatic CSML-0 cells.

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Cell Line	³ (H)Thymidine		Cells/Dish	DNA Synthesis/Cell
	Incorporation (dpm)			
CSML-0	6428	1.6×10^6	4.0×10^{-3}	
CSML-100	3700	1.1×10^6	3.3×10^{-3}	

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In particular, only 1.1×10^6 CSML-100 cells are observed per dish, whereas 1.6×10^6 CSML-0 cells are observed. Since 1×10^6 cells of both type were plated, the CSML-100 cell growth was only about one-sixth that of the CSML-0 cell growth. Fig. 11 further illustrates that the growth of CSML-100 cells from a 2-day to at least a 5-day period is less than that of CSML-0 cells.

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EXAMPLE 15mts-1 mRNA Can Be Detected by Hybridization
of mts-1 Antisense Probes to Tissue Sections

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Methods

Mouse embryonic trophoblast cells express mts-1. To illustrate the efficacy of mts-1 nucleic acid probes for detection of mts-1 mRNA in tissue sections, frozen sections of an 8 day mouse embryo were obtained. Sections were placed onto a standard microscope slide and fixed for 5 min. with 3% formaldehyde, 0.1M phosphate buffer, pH 7.2.

Sense and antisense mts-1 riboprobes were prepared by in vitro transcription from a pGEM-2-mts-1 vector containing the 3'untranslated region of mts-1 using T₇ and T₃ RNA polymerases according to the manufacturers direction. Transcription was with ³H-UTP (45μG, Amersham).

Prior to hybridization, slides were acetylated with 0.25% (v/v) acetic anhydride in 0.1M triethanolamine for 10 minutes at room temperature. The sections were rinsed in 2X SSC and dehydrated through an ethanol series (30%, 50%, 70%, 85%, 95%, 99%, 99%).

To the dried sections, 20μl of hybridization solution (0.3M NaCl, 20mM Tris pH 8, 1mM EDTA, 50% formamide, 10% dextran sulfate, 1X Denhardt's, 500μg/ml yeast RNA) containing ~10⁴ cpm/μl ³H sense or antisense probe, preheated to 80°C, was applied and secured with a coverslip. The slides were immersed in mineral oil and incubated at 45°C for ~12 hours. Excess mineral oil was removed from slides, and slides were washed through

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1 chloroform 3 times. Slides were next rinsed 3 x 5 in 4X
SSC to remove coverslips. RNase digestion (20µg/ml
RNase A, 1U/ml RNase T₁) in 0.5M NaCl, 10mM Tris pH 8.0,
1mM EDTA) was done for 30 minutes at 37°C. Slides were
5 washed once in RNase buffer for 30 minutes, 37°C; then
in 4 liters 2X SSC for 30 minutes at room temperature;
next in 0.1X SSC at 55°C for 30 minutes; finally in 4
liters 0.1X SSC at room temperature for 30 minutes.
Slides were dehydrated through an ethanol series
10 containing 300mM ammonium acetate. Once slides were
dry, they were dipped in NTB-2 emulsion (Kodak) [diluted
1:1 with 600mM ammonium acetate] and autoradiographed
for 4 weeks. Slides were developed in D-19 2.5 minutes,
fixed in 2% acetic acid 30 seconds, fixed for 5 minutes
15 and rinsed in water for 30 minutes. Finally, sections
were counter stained, cover slipped and photographed
using dark-field illumination.

Results

Hybridized mts-1 probe was detected in mouse
20 trophoblast cells only when the mts-1 probe was an
antisense probe (Fig. 12a). The sense mts-1 probe gave
rise to no signal (Fig. 12b). These data indicate that
an antisense mts-1 probe can be used to detect mts-1
mRNA in tissue sections.

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EXAMPLE 16Chicken Anti-*mts-1* Antibody Detects *mts-1* Protein by Western Blot and Immunohistochemistry

5

Methods:

The chicken anti-*mts-1* antibody (α -*mts-1*) was prepared as described in Example 9.

10 Lysates of CSML-0 and CSML-100 cells were electrophoresed on 12% SDS-PAGE gels and transferred to polyvinylidene difluoride (PVDF) membranes for Western blot analysis. Membranes containing 3 μ g purified *mts-1* were incubated with anti-*mts-1* antibody (1:2000) for 3 hours at room temperature and then with anti-chicken IgG-HRP at room temperature for 2 hours. Signal was
15 detected with diaminobenzene (DAB).

To test the specificity of the α -*mts-1* antibody, membranes containing CSML-100 proteins were probed with α -*mts-1* in the presence and absence of
20 260 ng free recombinant *mts-1* protein. Membranes containing 3 μ g purified *mts-1* were treated as above but upon addition of the primary antibody (α -*mts-1*) 13 μ g of purified free *mts-1* was added.

Frozen mouse spleens were sectioned and fixed
25 onto glass slides. Sections were probed with a 1:1000 dilution of α -*mts-1* in PBA according to the method of Harlow et al. To test the specificity of the α -*mts-1* antibody, 130 ng free *mts-1* protein was applied to one series of slides along with the diluted α -*mts-1*
30 antibody.

The α -*mts-1* antibody was deemed specific for *mts-1* protein when free *mts-1* effectively eliminated

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- 1 binding of α -mts-1 to mts-1 Western blots or mouse
spleen sections.

Results:

- 5 Fig. 13 depicts a Western blot of CSML-0 (Lane
1) and CSML-100 (Lanes 2 and 3) cell lysates. Lanes 1
and 2 were probed with α -mts-1 antibody without added
free mts-1 protein. As illustrated, a 10-12 Kd mts-1
10 protein is expressed in CSML-100 cells (Lane 2) but not
in CSML-0 cells (Lane 1). Moreover, 260 ng free mts-1
protein effectively eliminated antibody binding to the
CSML-100 cell lysate in Lane 3. Therefore, the α -mts-1
antibody is highly specific for mts-1 protein.

- 15 Similarly, mts-1 protein is detected in frozen
mouse spleen sections (Fig. 14a) and α -mts-1 antibody
binding on such tissue sections is eliminated when free
mts-1 protein is applied to the sections with the
antibody (Fig. 14b).

- 20 Accordingly, mts-1 protein can readily be
detected on Western blots and on tissue sections using
the α -mts-1 antibody.

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EXAMPLE 17Low Molecular Weight mts-1 Protein is Found
Only in Serum From Animals with Metastatic CancerMethods:

Mouse Studies: CSML-0 and CSML-100 cells were injected intravenously into the tail veins of A/J mice at 1×10^7 cells per mouse (Fig. 15a, Lane 2) or 1×10^6 cells per mouse (Fig. 15b, Lane 2). After three weeks the mice were sacrificed, their lungs examined for the presence of metastasis, and their blood drawn. Blood was allowed to clot at room temperature for one hour and was then microfuged to isolate sera. The sera samples were loaded at 100 μ g per lane on a 13% SDS-PAGE gel (Fig. 15a) or a 13% Tris-Tricine gel (Fig. 15b). The proteins were then transferred to PVDF membranes and probed with a 1:1000 dilution of α -mts-1 antibody and a horse-radish peroxidase conjugated secondary antibody.

mts-1 is expressed in T lymphocytes and activated macrophages. To test whether the detected mts-1 protein resulted from lysis of normal blood cells including T lymphocytes and macrophages, whole mouse blood was lysed and probed with α -mts-1 antibody in a western blot analysis. Whole blood was taken from a normal mouse, lysed in a triton-X100 solution and electrophoresed on a 13% Tris-Tricine gel. A PVDF membrane blot of the gel was prepared and probed as above.

To determine whether the presence of mts-1 in sera is simply due to a chronic immune response which might increase the number of T lymphocytes and activated

-88-

1 macrophages, mice were injected with salmonella LPS over
an extended time period to induce a chronic immune
response. Sera were drawn and western analysis was
performed as described above.

5 Human Studies: Serum samples were obtained
from normal women and patients with breast carcinomas or
advanced malignant lymphomas. 150 µg of each serum
sample was run on a 12% SDS-PAGE gel. The proteins were
transferred to PVDF membranes and the membranes were
10 probed with a 1:1000 dilution of α -mts-1 and then with a
1:1000 dilution of the secondary antibody (rabbit anti-
chicken IgG-HRP). The reaction was developed with a DAB
solution.

15 Results:

Mouse Studies: Three weeks after intravenous
injection, the mice receiving CSML-100 looked very sick
and had breathing difficulties. Western analysis of
sera from injected and non-injected animals indicated
20 that only those mice receiving CSML-100 cells had a 10-
12 Kd mts-1 protein (Figs. 15a and 15b , Lane 2).
Injection of as little as 10^5 CSML-100 cells three weeks
prior to western analysis produced a positive serum
response. The lungs of those mice injected with either
25 10^5 or 10^6 CSML-100 cells had extensive metastasis.

 The α -mts-1 antibody detected a high molecular
weight band in all samples on the western blot.
However, addition of free mts-1 protein to the Western
blot when incubating with the α -mts-1 antibody did not
30 eliminate the signal from the high molecular weight
band. Only the lower molecular weight band found in
CSML-100 injected mice was eliminated by competing free

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-89-

1 mts-1 protein. Therefore, only the lower molecular
weight band is mts-1 protein. The higher molecular
weight band may be an abundant serum protein which
cross-reacts with the α -mts-1 polyclonal antibody.

5 Fig. 15c illustrates that the mts-1 protein
detected in serum is not a normal component of whole
blood and is not a result of a chronic immune response.
The mts-1 protein is not detected in lysed whole blood
cells (Fig. 15c, Lanes 1-4 containing 5, 10, 20 and
10 25 μ l lysed whole blood). However, mts-1 was detected
in similarly treated CSML-100 cells which were provided
as a positive control (Fig. 15c, Lane 5).

Fig. 15d illustrates that the mts-1 protein
detected in sera of metastatic cancer patients is not
15 due to a chronic immune response induced by salmonella
LPS over an extended period of time. The mts-1 protein
could not be detected in the 75 μ g, 100 μ g or 150 μ g of
sera from chronically immunologically stimulated mice
(Fig. 15d, Lanes 1-3).

20 Accordingly, a 10-12 Kd mts-1 protein can be
detected in sera of mice with metastatic cancer. No
mts-1 protein is detected in the serum of control mice.

Human Studies: Fig. 16 illustrates that mts-1
25 protein can be detected only in sera from patients known
to have metastatic cancer. An approximate 27 Kd mts-1
protein could be detected in serum from a patient with
metastatic breast cancer (Fig. 16, Lane 6) and in two
patients with metastatic lymphomas (Fig. 16, Lanes 5
30 and 7).

However, no such 27 Kd mts-1 protein was
detected in serum from a normal patient (Fig. 16, Lane

-90-

1 3) or in serum from patients with non-metastatic breast
cancer (Fig. 16, Lane 1) or non-metastatic lymphomas
(Fig. 16, Lanes 2 and 4).

5 The higher molecular weight band apparent in
Figs. 16a-d is not mts-1 protein. In particular, when
the Western blot is probed with α -mts-1 antibody in the
presence of free mts-1 protein, only the 27 Kd protein
band disappears. Free mts-1 protein does not eliminate
10 the high molecular weight signal. Therefore, the α -mts-1
polyclonal antibody may cross-react with an abundant
serum protein. Such cross-reactivity can be eliminated
by, for example, using an antibody directed against
human mts-1 protein (α -mts-1 is directed against mouse
15 mts-1 protein) or by employing highly specific
monoclonal antibodies prepared as described in Example
10.

 Accordingly, mts-1 protein is detectable only
in sera from patients with metastatic cancer. The mts-1
protein cannot be detected in the serum of normal
20 patients or in the serum of patients with non-metastatic
cancer. Antibodies directed against mts-1 protein can
therefore be used in a simple serum immunoassay to
diagnose and detect metastatic cancer in patients.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Zain, Sayeeda
Lukanidin, Eugene

(ii) TITLE OF INVENTION: DIAGNOSIS OF METASTATIC CANCER BY
THE MTS-1 GENE

(iii) NUMBER OF SEQUENCES: 9

(iv) CORRESPONDENCE ADDRESS:

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(B) STREET: 400 Garden City Plaza
(C) CITY: Garden City
(D) STATE: New York
(E) COUNTRY: United States
(F) ZIP: 11530

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER:
(B) FILING DATE:
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: DiGiglio, Frank S.
(B) REGISTRATION NUMBER: 31,346
(C) REFERENCE/DOCKET NUMBER: 7879ZY

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (516) 742-4343
(B) TELEFAX: (516) 742-4366
(C) TELEX: 230 901 SANS UR

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 303 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

- 92 -

(11) MOLECULE TYPE: cDNA

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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ATGGCGTGCC CTCTGGAGAA GGCCCTGGAT GTGATGGTGT CCACCTTCCA CAAGTACTCG      60
GGCAAAGAGG GTGACAAGTT CAAGCTCAAC AAGTCAGAGC TAAAGGAGCT GCTGACCCGG      120
GAGCTGCCCCA GCTTCTTGGG GAAAAGGACA GATGAAGCTG CTTTCCAGAA GCTGATGAGC      180
AACTTGGACA GCAACAGGGA CAACGAGGTG GACTTCCAAG AGTACTGTGT CTTCCTGTCC      240
TGCATCGCCA TGATGTGTAA CGAATTCTTT GAAGGCTTCC CAGATAAGCA GCCCAGGAAG      300
AAA                                                                303

```

(2) INFORMATION FOR SEQ ID NO:2:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 101 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: protein

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```

Met Ala Cys Pro Leu Glu Lys Ala Leu Asp Val Met Val Ser Thr Phe
 1              5              10              15
His Lys Tyr Ser Gly Lys Glu Gly Asp Lys Phe Lys Leu Asn Lys Ser
          20              25              30
Glu Leu Lys Glu Leu Leu Thr Arg Glu Leu Pro Ser Phe Leu Gly Lys
          35              40              45
Arg Thr Asp Glu Ala Ala Phe Gln Lys Leu Met Ser Asn Leu Asp Ser
          50              55              60
Asn Arg Asp Asn Glu Val Asp Phe Gln Glu Tyr Cys Val Phe Leu Ser
          65              70              75              80
Cys Ile Ala Met Met Cys Asn Glu Phe Phe Glu Gly Phe Pro Asp Lys
          85              90              95
Gln Pro Arg Lys Lys
          100

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-93-

(2) INFORMATION FOR SEQ ID NO:3:

- (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 579 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(11) MOLECULE TYPE: cDNA

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:3:

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GGCAGTTGAG GCAGGAGACA TCAAGAGAGT ATTTGTGCCC TCCTCGGGTT TTACCTTCCA      60
GCCGAGATTC TTCCCTCTC TACAACCTC TCTCCTCAGC GCTTCTTCTT TCTTGGTTTG      120
ATCCTGACTG CTGTCATGGC GTGCCCTCTG GAGAAGGCCC TGGATGTGAT GGTGTCCACC      180
TTCCACAAGT ACTCGGGCAA AGAGGGTGAC AAGTTCAAGC TCAACAAGTC AGAAGTAAAG      240
GAGCTGCTGA CCCGGGAGCT GCCCAGCTTC TTGGGGAAAA GGACAGATGA AGCTGCTTTC      300
CAGAAGCTGA TGAGCAACTT GGACAGCAAC AGGGACAACG AGGTGGACTT CCAAGAGTAC      360
TGTGTCTTCC TGTCTGTCAT CGCCATGATG TGTAACGAAT TCTTTGAAGG CTTCCCAGAT      420
AAGCAGCCCCA GGAAGAAATG AAAACTCCTC TGATGTGGTT GGGGGGTCTG CCAGCTGGGG      480
CCCTCCCTGT CGCCAGTGGG CACTTTTTTT TTTCCACCCT GGCTCCTTCA GACACGTGCT      540
TGATGCTGAG CAAGTTCAAT AAAGATTCTT GGAAGTTTA      579

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(2) INFORMATION FOR SEQ ID NO:4:

- (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 10 amino acids
 (B) TYPE: amino acid
 (C) STRANDEDNESS: double
 (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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Ala Cys Pro Leu Glu Lys Ala Leu Asp Val
1           5           10

```

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(2) INFORMATION FOR SEQ ID NO:5:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 16 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:5:

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1 5 10 15

(2) INFORMATION FOR SEQ ID NO:6:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:6:

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1 5 10

(2) INFORMATION FOR SEQ ID NO:7:

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(11) MOLECULE TYPE: DNA (genomic)

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asn Glu Phe Phe Glu Gly Phe Pro Asp Lys Gln Pro Arg Lys Lys
1 5 10 15

- 95 -

(2) INFORMATION FOR SEQ ID NO:8:

- (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(11) MOLECULE TYPE: cDNA

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ATGGCGTGCC CTCTGGAGAA G

21

(2) INFORMATION FOR SEQ ID NO:9:

- (1) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 21 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(11) MOLECULE TYPE: cDNA

(x1) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TTTCTTCCTG GGCTGCTTAT G

21

WHAT IS CLAIMED IS:

1. An isolated nucleic acid encoding an antigenic fragment of human mts-1 protein comprising at least 4 amino acids.
2. The isolated nucleic acid of Claim 1 wherein said nucleic acid is genomic DNA, cDNA or RNA.
3. An isolated nucleic acid encoding a human mts-1 protein comprising the nucleotide sequence set forth in SEQ ID NO: 3.
4. A replicable expression vector comprising the nucleic acid of Claim 1 operably linked to a nucleotide sequence capable of effecting expression of a polypeptide encoded by said nucleic acid.
5. An isolated antigenic fragment of human mts-1 polypeptide.
6. A host cell comprising the nucleic acid of any one of Claims 1-3.
7. A host cell according to Claim 6 wherein said cell is yeast, a bacterium, a mammalian cell or an insect cell.
8. A method for diagnosing metastatic cancer which comprises contacting serum from an individual to be tested for said cancer with an antibody reactive with a mammalian mts-1 protein or an antigenic fragment

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thereof, for a time and under conditions sufficient to form an antigen-antibody complex, and detecting said antigen-antibody complex.

9. The method of Claim 8 wherein said mts-1 protein or an antigenic fragment thereof is human, rat or mouse.

10. The method of Claim 8 wherein said cancer is lung, liver, kidney, thyroid, breast, leukemic, pancreatic, endometrial, ovarian, cervical, skin, colon, or lymphoid cancer.

11. An isolated continuous cultured cell line capable of expressing mts-1 protein or an antigenic fragment thereof.

12. A cell line according to Claim 11 expressing the mts-1 protein comprising SEQ ID NO:2.

13. A cell line according to Claim 11 wherein the mts-1 protein or antigenic fragment thereof is detectable with anti-mts-1 antibodies.

14. A method of treatment of cancer comprising therapeutically administering an oligonucleotide capable of binding to an mts-1 mRNA.

15. The method of Claim 14 wherein said oligonucleotide comprises at least 10 nucleotides of an antisense strand of SEQ ID NO:1 or SEQ ID NO:3.

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16. The method of Claim 14 wherein said cancer is selected from the group consisting essentially of lung, liver, kidney, thyroid, breast, leukemic, pancreatic, endometrial, ovarian, cervical, skin, colon or lymphoid cancer.

17. A pharmaceutical composition comprising a therapeutically-effective amount of an antibody reactive with a mammalian mts-1 protein and a pharmaceutically acceptable carrier therefor.

18. The pharmaceutical composition of Claim 17 wherein said pharmaceutically effective amount of said antibody is about 0.5 µg to 2000 mg per kilogram of body weight per day.

19. A method of inhibiting metastasis in a cancerous cell which comprises providing to said cancerous cell a nucleic acid encoding an antisense mts-1 nucleotide sequence.

20. A method of inhibiting metastasis in a cancerous cell which comprises providing to said cancerous cell an expression vector comprising a nucleic acid encoding an antisense nucleotide sequence for a mammalian mts-1 operably linked to a segment of said vector which can effect expression of an antisense mts-1 RNA.

21. An isolated antisense nucleic acid of human mts-1.

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22. The isolated antisense nucleic acid of Claim 21 which is RNA.

23. The isolated antisense nucleic acid of Claim 21 comprising at least 10 nucleotides of an antisense strand of SEQ ID NO:1 or SEQ ID NO:3.

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FIG. 1

ATG-GCG-TGC-CCT-CTG-GAG-AAG-GCC-CTG-GAT-GTG-ATG-GTG-TCC-ACC-
TTC-CAC-AAG-TAC-TCG-GGC-AAA-GAG-GGT-GAC-AAG-TTC-AAG-CTC-AAC-
AAG-TCA-GAG-CTA-AAG-GAG-CTG-CTG-ACC-CGG-GAG-CTG-CCC-AGC-TTC-
TTG-GGG-AAA-AGG-ACA-GAT-GAA-GCT-GCT-TTC-CAG-AAG-CTG-ATG-AGC-
AAC-TTG-GAC-AGC-AAC-AGG-GAC-AAC-GAG-GTG-GAC-TTC-CAA-GAG-TAC-
TGT-GTC-TTC-CTG-TCC-TGC-ATC-GCC-ATG-ATG-TGT-AAC-GAA-TTC-TTT-
GAA-GGC-TTC-CCA-GAT-AAG-CAG-CCC-AGG-AAG-AAA

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FIG. 2

1	14
Met-Ala-Cys-Pro-Leu-Glu-Lys-Ala-Leu-Asp-Val-Met-Val-Ser-	
Thr-Phe-His-Lys-Tyr-Ser-Gly-Lys-Glu-Gly-Asp-Lys-Phe-Lys-	
Leu-Asn-Lys-Ser-Glu-Leu-Lys-Glu-Leu-Leu-Thr-Arg-Glu-Leu-	
Pro-Ser-Phe-Leu-Gly-Lys-Arg-Thr-Asp-Glu-Ala-Ala-Phe-Gln-	
Lys-Leu-Met-Ser-Asn-Leu-Asp-Ser-Asn-Arg-Asp-Asn-Glu-Val-	
Asp-Phe-Gln-Glu-Tyr-Cys-Val-Phe-Leu-Ser-Cys-Ile-Ala-Met-	
Met-Cys-Asn-Glu-Phe-Phe-Glu-Gly-Phe-Pro-Asp-Lys-Gln-Pro-	
Arg-Lys-Lys	

3 / 20

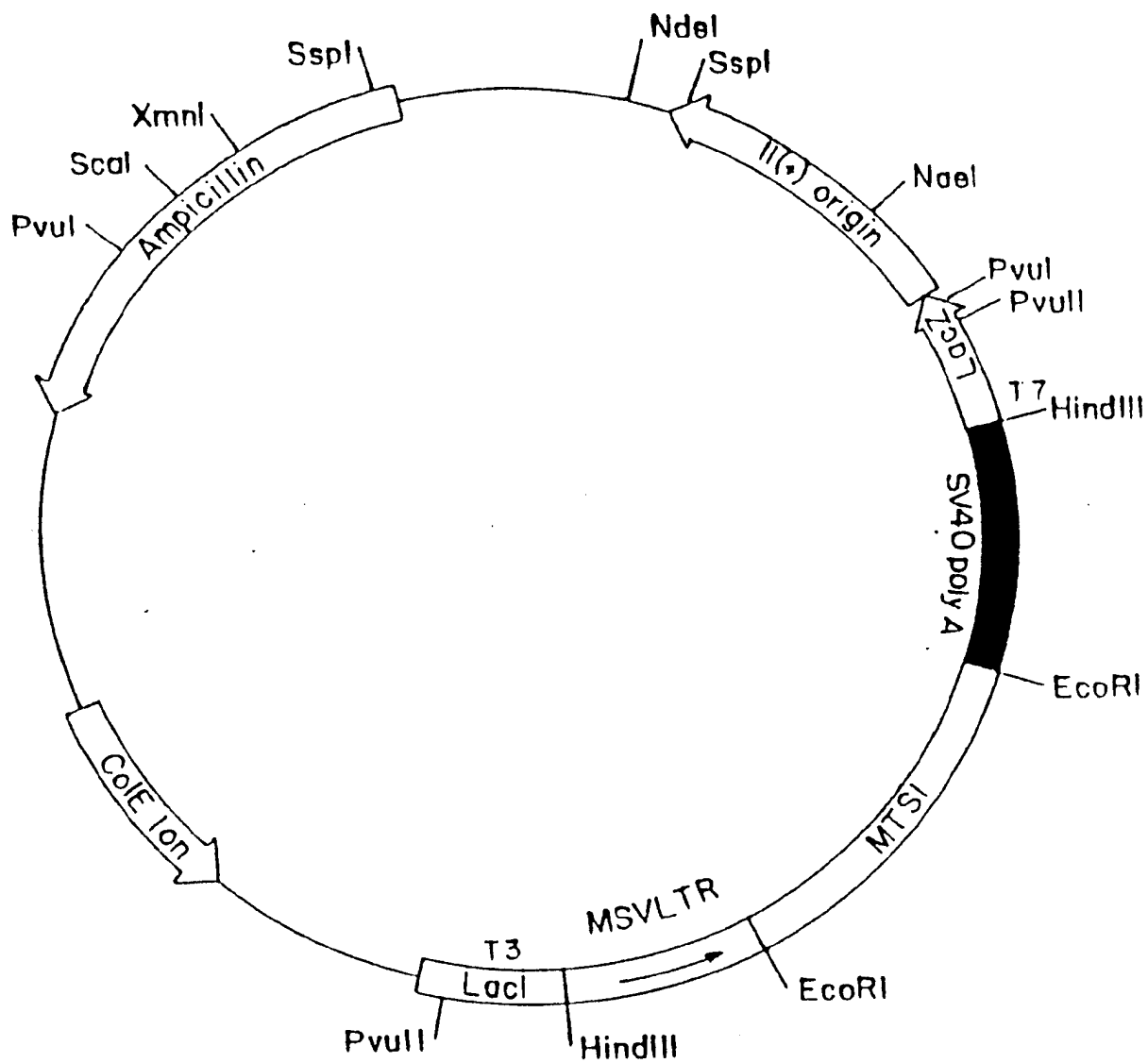


FIG. 3

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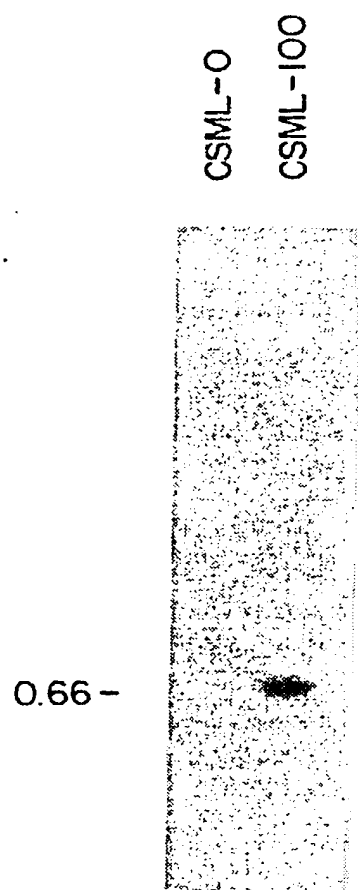


FIG. 4

5/20.

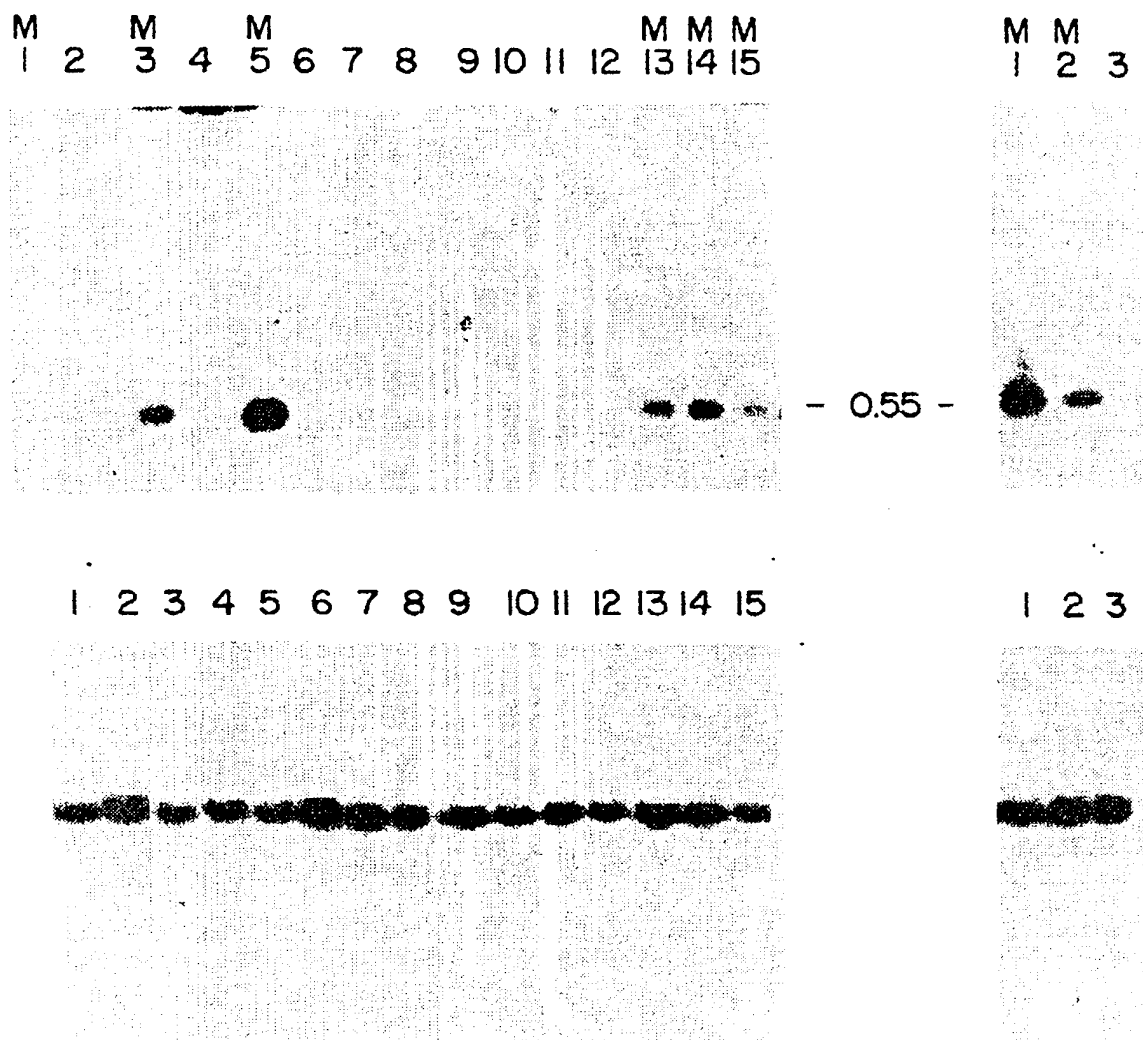


FIG. 5

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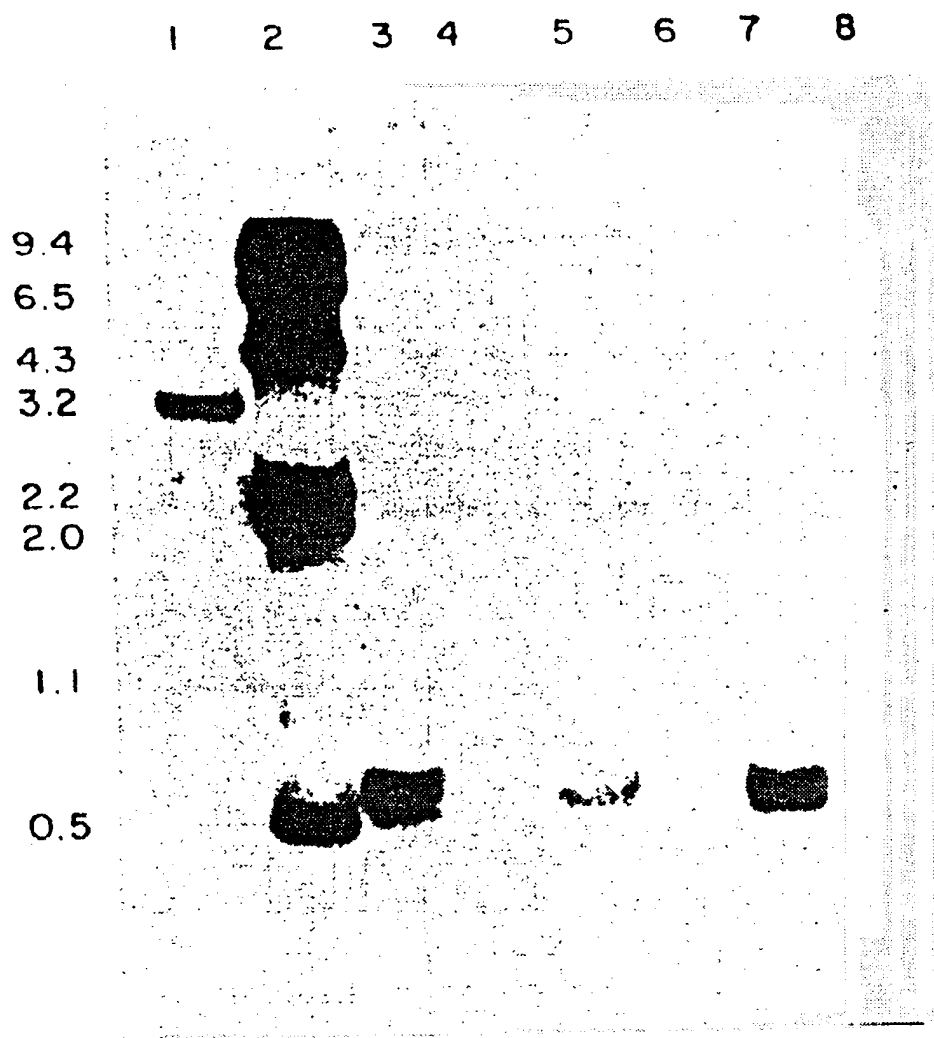


FIG. 6

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FIG. 7A

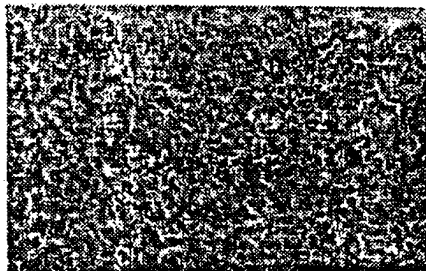


FIG. 7B



FIG. 7C



FIG. 7D

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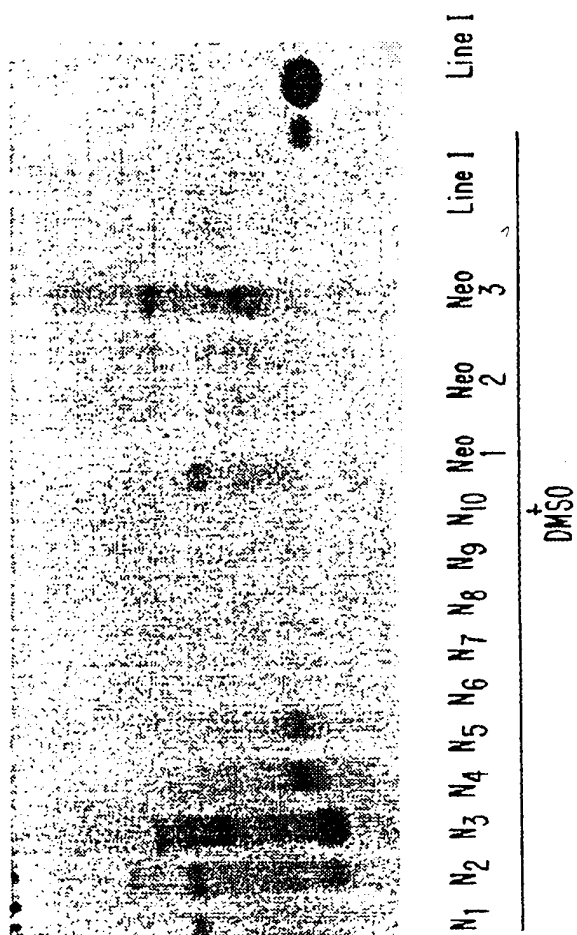


FIG. 8

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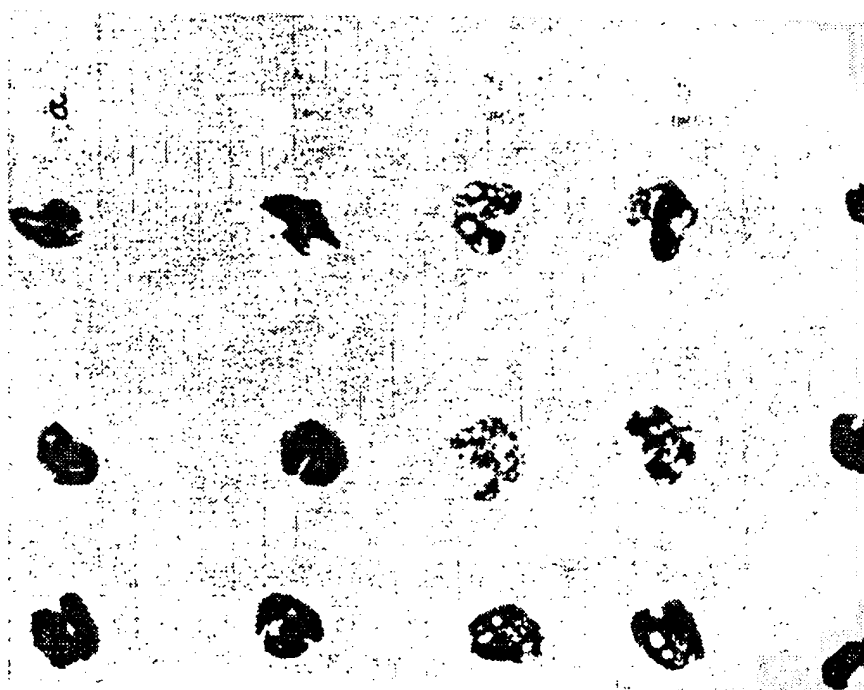
FIG. 9B

FIG. 9D

FIG. 9A

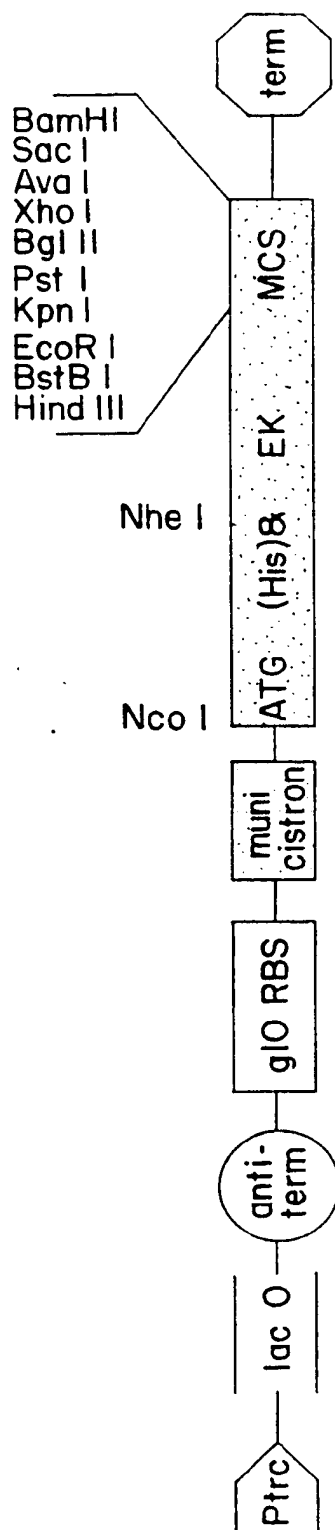
FIG. 9C

FIG. 9E



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FIG.10A



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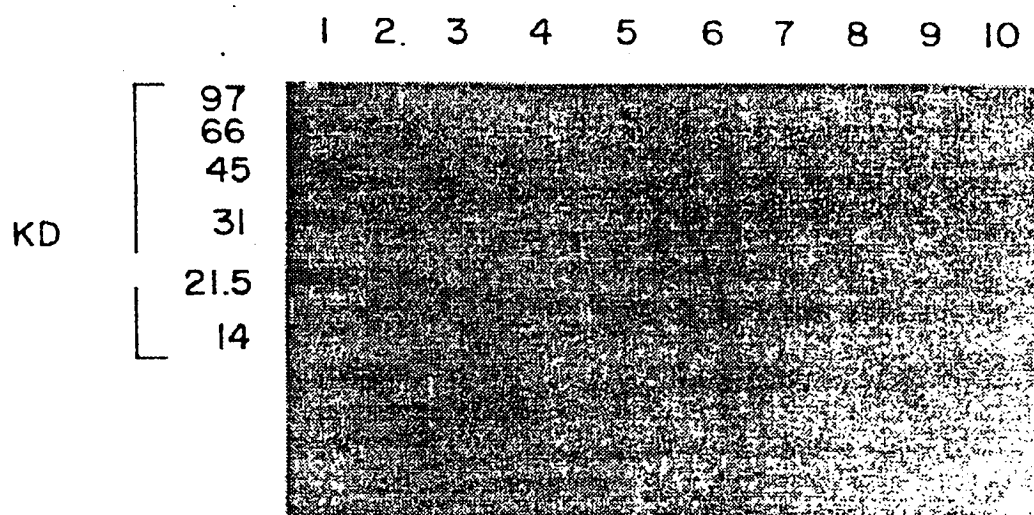
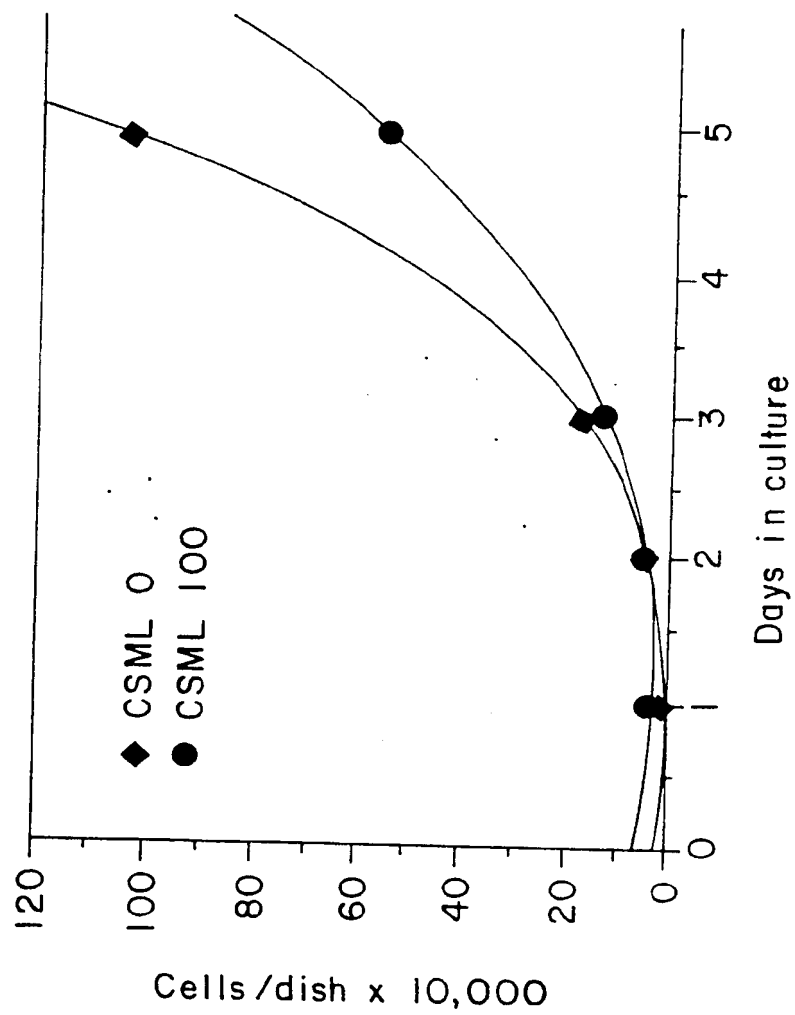


FIG. 10B

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FIG. II



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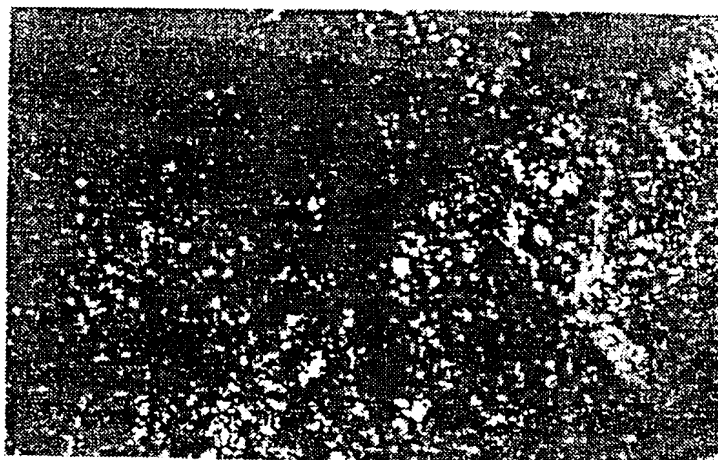


FIG. 12 A

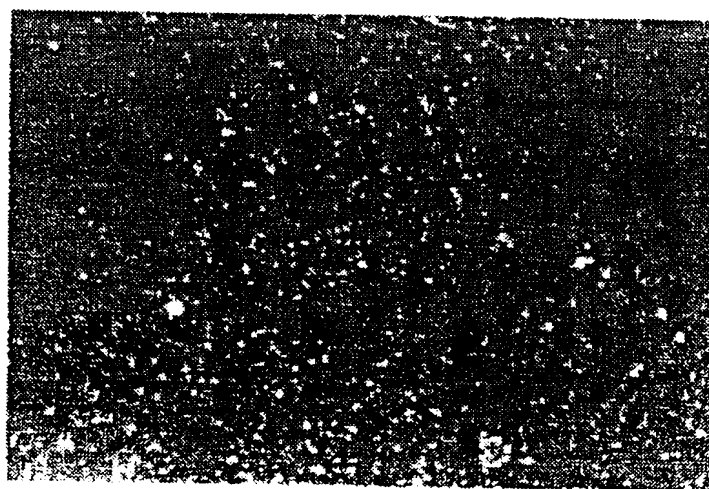


FIG. 12 B

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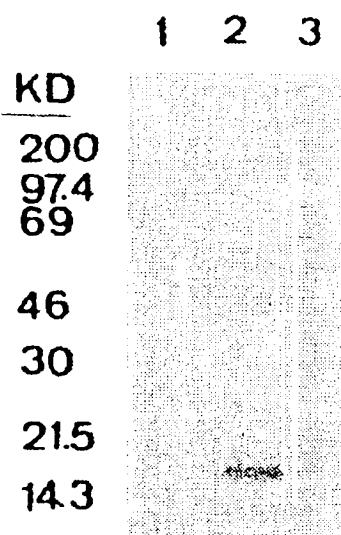


FIG. 13

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FIG. 14A

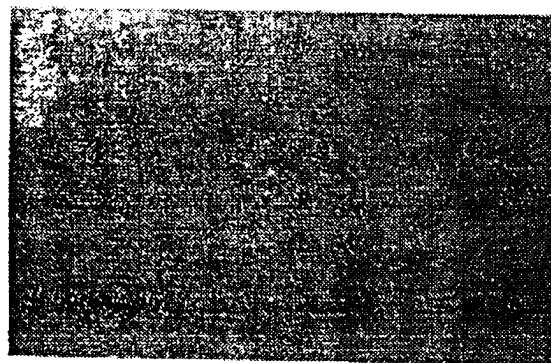


FIG. 14B

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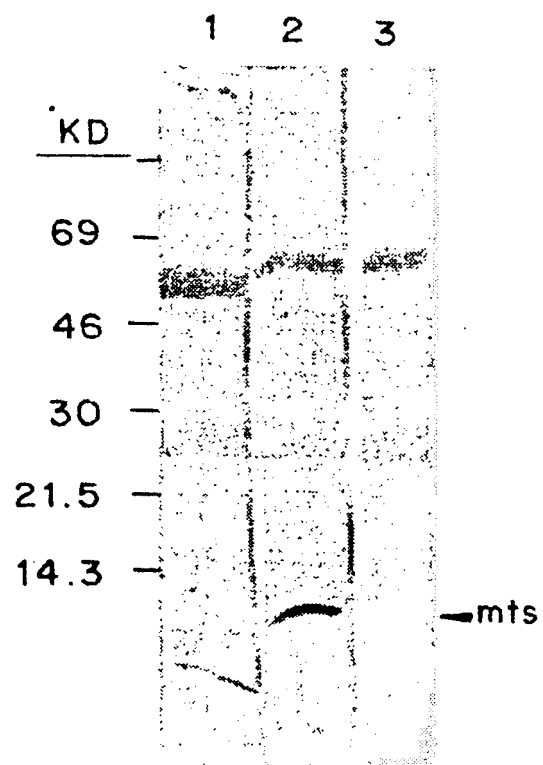


FIG. 15A

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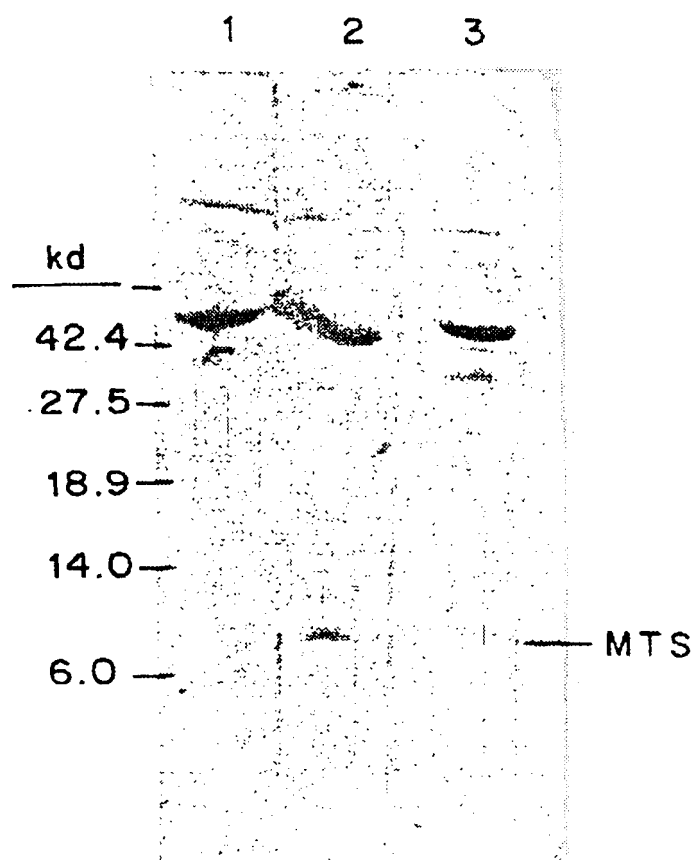


FIG. 15B

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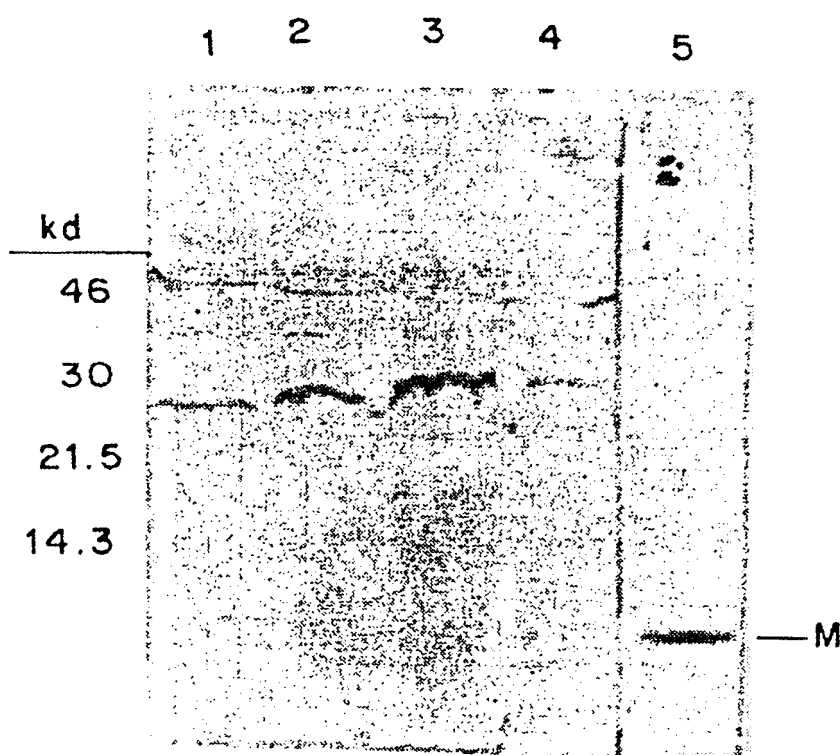


FIG. 15C

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FIG. 15D

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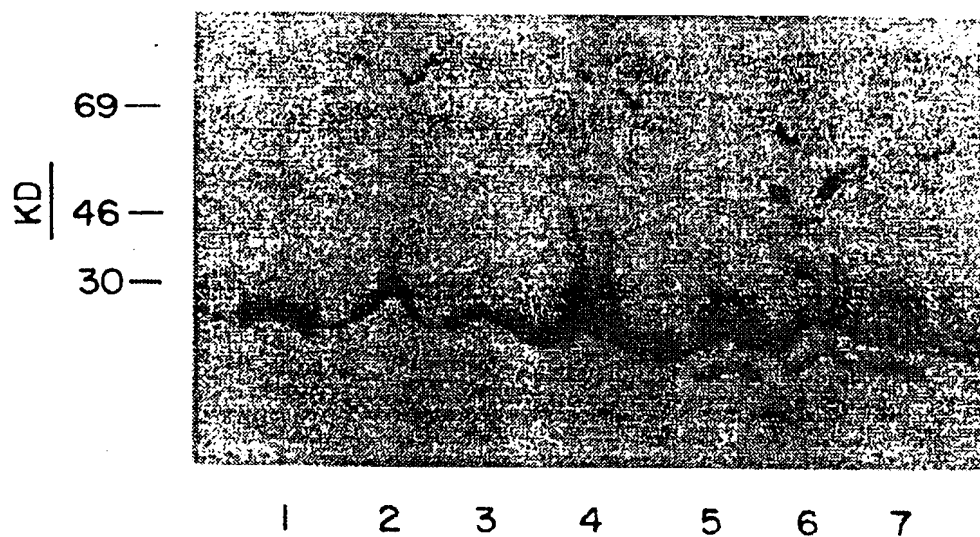


FIG. 16

INTERNATIONAL SEARCH REPORT

Int. Application No

PCT/US 95/01214

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C12N15/63 C07K14/47 C12N5/10 C12N1/19
 C12N1/21 G01N33/574 A61K48/00 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07K C12N G01N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>WO,A,92 00757 (RESEARCH CORPORATION TECHNOLOGIES, INC.) 23 January 1992</p> <p>see page 3, line 26 - page 4, line 21 see page 6, line 20 - page 7, line 32 see page 8, line 23 - page 11, line 23 see page 12, line 10 - page 13, line 18 see page 15, line 10 - page 16, line 15 see page 20, line 15 - page 21, line 22 see page 26, line 27 - page 28, line 4 see page 29, line 18 - line 31; examples 4-13</p> <p style="text-align: center;">--- -/--</p>	<p>1,2, 4-13,17, 18</p>

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search

19 May 1995

Date of mailing of the international search report

31-05-1995

Name and mailing address of the ISA

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Authorized officer

Montero Lopez, B

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 95/01214

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	GENE, vol. 87, no. 2, 15 March 1990 pages 219-223, E.M. TULCHINSKY ET AL. 'Structure of gene mts-1, transcribed in metastatic mouse tumor cells' see abstract see page 221, left column, paragraph 1 - page 222, left column, paragraph 1 see page 222, left column, paragraph 4 - right column, last paragraph ---	1,2,4,6, 7,11
X	GENES & DEVELOPMENT, vol. 3, 1989 pages 1086-1093, ALEXANDER EBRALIDZE ET AL. 'Isolation and characterization of a gene specifically expressed in different metastatic cells and whose deduced gene product has a high degree of homology to a Ca ²⁺ -binding protein family' see abstract see page 1087, left column, paragraph 2 - last paragraph -----	1,2

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 95/01214

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark : Although claims 14-16 and 19-20, as far as concerning an in-vivo method, are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 95/01214

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9200757	23-01-92	CA-A- 2086829	10-01-92
		EP-A- 0566571	27-10-93
		JP-T- 6500018	06-01-94
